Activity patterns and organization within ant nests

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Abstract

I develop an automated technique for measuring positional change, and employ this system to measure short term patterns of activity in laboratory nests of the ant species *Leptothorax acervorum* and *L. tubero-interruptus*. Individuals of these species appear to synchronize their activity such that cycles in colony activity level occur at approximately 20 minute intervals for *L. acervorum* and 35 minutes for *L. tubero-interruptus*.

Using a combination of manual and automated techniques, I test a number of models designed to account for short term activity cycles in ant nests. The results do not support the predictions of Goss and Deneubourg (1988) or Hemerik et al. (1990), but are broadly in agreement with the predictions of Tofts (1990a).

I investigate the behaviour and activity patterns of individuals of *L. acervorum*, and demonstrate a division of labour in this species. The two task groups indentified appear to differ in their activity profiles, cycling in activity at different rates, and leading to a complex pattern of activity at the colony level.

I investigate the effect of food deprivation on colony and individual activity, and demonstrate that colony activity cycles are maintained, although individuals shift their activity profiles as food deprivation continues.

I employ automated techniques to investigate the strength of coupling of activity levels between different regions of the nest, and to investigate patterns of activity in differing physical designs of nests.

I discuss a number of models designed to account for the adaptive significance of the observed patterns of activity in ant nests.

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Contents

Chapter 1

Introduction

1.1 Approaches to the study of social insects

Social insects have been utilized as models for considering a number of important problems in evolution and behavioural ecology. Much of the attention has focused on the evolution of eusociality (Evans, 1958, 1977; Michener, 1956, 1974; Wilson, 1971) with reference to kin selection (Hamilton, 1963, 1964; West-Eberhard, 1975, 1981), sex ratio theory (Trivers and Hare, 1976; Alexander and Sherman, 1977; Nonacs, 1986), the occurence of polygyny (Hölldobler and Wilson, 1977; Buschinger, 1968a; Bourke, 1991), and social parasitism (Bourke and Franks, 1991; Buschinger, 1965, 1990; Wilson, 1975; Alloway, 1979, 1980).

Behaviour of individuals has often been studied within the context of such evolutionary problems, with reference to factors thought to promote the success of eusocial species, such as division of labour (Oster and Wilson, 1978; Wilson, 1968), and possible conflicts that occur between members of a colony under selection to maximize their inclusive fitness (Bourke, 1988b, 1991; Trivers and Hare, 1976; Cole, 1981; Franks and Scovell, 1983).

The strong emphasis on reductionism has yielded many important contributions to our understanding of conflict and cooperation at the level of individuals, but owing to the nature of questions addressed we appear to be more familiar with the "anatomy" of colonies as static objects than with the dynamics of interactions among individuals that presumably lead to functional organization of the colony as a whole. For instance, division of labour has generally been considered as a static phenomenon; individuals being classified to one of a number of castes depending on their behavioural repertoire (for instance Wilson, 1976; Wilson and Fagen 1974; Fagen and Goldman, 1977). As Gordon (1985) points out, few studies have considered the dynamics of labour division, namely mechanisms underlying short term choice of task, or changes in caste profile if the colony environment is manipulated (but see Calabi 1988; Gordon 1986, 1988b).

The logical similarity between social insect colonies and functionally organized units such as organisms has been recognized for some time (Wheeler, 1911; Sturtevant, 1938; recently see Wilson and Sober, 1989). Such holistic approaches to colony function have suffered from two drawbacks. Firstly, there are difficulties in designing a coherent research programme, in terms of testable models, without recourse to reductionist methodologies (Popper 1972, 1974; for recent debate see Lakatos and Musgrave, 1970). Secondly, holism in function has been linked to holism in selection. Evolution occurs as a result of selection between genes that reside in individual organisms (Williams, 1966; Dawkins, 1976). The extent to which selection can occur between higher levels of organization, such as colonies, populations and species is still under discussion (Wilson and Colwell, 1981; Colwell 1981; Sober, 1984, 1987; Grafen, 1984; Maynard Smith, 1987a, 1987b), although the original group selection arguments of Wynne Edwards (1962) have largely been rejected. Although selection may only occur between genes, functional organization occurs at higher levels, namely organisms, and to some extent

certain groups of organisms, among which social insect colonies would appear to be a good example (Gould and Lewontin, 1979; Wilson and Sober, 1989). With regard to behavioural (functional) organization then, a holistic approach need not be at odds with reductionism in selection.

Unfortunately the holistic approach to viewing colonies as "superorganisms" has historically yielded little more than the persuasive and provocative analogy on which it is based. Although E.O. Wilson (1985a) developed this concept to allow division of labour to be considered analogous to cell differentiation in development, and Lumsden (1982) used the superorganism approach to consider problems of homeostasis, our understanding of integration and organization within colonies is still scant and limited to specific examples owing to the paucity of empirical data. A purely holistic approach to colonies would seem equally unable to provide insight into colony behavioural organization as one that considers individuals as static, independent entities. What is required is an amalgamation of the two approaches, whereby colonies can be behaviourally "dissected" into simpler units, but interactions between units and change of units is retained as a focus of investigation.

Although conflicts appear to occur between individuals within the colony (Franks and Scovell, 1983; Bourke, 1988b; Choe, 1988), to a large extent many of the problems that individuals are engaged in solving are problems at the colony level. To whit, "problems" such as maintaining the nutritional status of the colony, colony defence and migration, efficient brood care and allocation of resources (individuals) among tasks, must be solved in order to allow (increased) production of sexuals. There is considerable evidence to suggest that in many instances individuals are not capable of solving such problems independently; solution comes as a byproduct of interactions between individuals. This evidence (and the con-

cept of self organization) is considered in the next section. To some extent, solution of these problems enables individuals to increase their inclusive fitness (i.e., barring conflicts over male production, sex ratio and polygyny), relative to individuals in a colony that fails to solve such problems. Certainly it is not the case that individual worker ants can be considered as individual solitary insects. Most importantly, they are either sterile or able to produce only unfertilized (male producing) eggs. Also, to varying extents it is likely that sensory, neuronal and anatomical apparatus has become degraded or specialized by evolution in a colony context such that workers are unable to function independently and solve the wide range of problems experienced by solitary organisms. Hence, although the approach outlined above is essentially reductionist in that we wish to consider the component elements (individuals) of a system (colony), it has a holistic aspect in that we have reason to believe that most problems occur, and are "solved", at the system level.

From a behavioural point of view, such a meeting of holism and reductionism requires the collection of empirical data concerning the simultaneous action of many individuals, so that we can investigate what individuals do in relation to what others do at the same time. Manual data collection on this scale is highly time consuming; the recent development of automated techniques for data recording, collection and analysis perhaps begins to make this aim feasible.

1.2 Aims

The aims of this work are primarily to extend the research into short term patterns of activity in leptothoracine ants.

As suggested earlier, the ability to collect sufficient data on the actions of many individuals simultaneously would be an important addition to traditional methods of studying social insect behaviour. In this thesis I develop an automated technique to measure the simple parameter of colony activity level, allowing efficient collection of data concerning a colony level phenomenon that is the result of the behaviour of many individuals. I attempt to use this system in conjunction with manual techniques to measure patterns of activity in ant nests, and to test models of these patterns.

In the remainder of this chapter I review aspects of leptothoracine biology, with particular reference to the behavioural ecology of *L. acervorum*, the primary experimental species.

In Chapter 2 I consider aspects of behavioural organization in ant colonies to clarify issues of information exchange that may be relevant to questions of function for short term activity cycles. In particular, I consider concepts of caste, and introduce experiments designed to elucidate the caste system in *L. acervorum.*

The development of an image processing system to measure colony activity levels is presented in Chapter 3, where I also consider the theoretical relationship between activity and differences between consecutive images (the basis of the automated system). Experimental tests of the system are also summarized.

In Chapter 4 I attempt to provide a description of activity patterns in laboratory colonies of *L.acervorum* and *L. tubero-interruptus*, using the automated system developed in Chapter 3. I also review the models designed to account for activity cycles in ant nests, and attempt to test them empirically using the data from image analysis. This chapter also considers aspects of model design and

testability.

I return to manual methods of measurement in Chapter 5, to assess the patterns of behaviour of individuals that lead to colony level patterns of activity. The data presented provides further tests of the assumptions underlying the various models.

A combination of automated measures of colony activity and manual measures of individuals is presented in Chapter 6 to assess the effect of prolonged food deprivation on activity patterns in *L.acervorum*. This data is employed to test the predictions of Hemerik et al. (1990), and the possible adaptive advantage of the observed responses are considered.

In Chapter 7 I present an automated technique to measure patterns of connectivity in activity within nests of differing physical design, and attempt to link patterns of short term activity to broader issues of behavioural organization in leptothoracine colonies. I attempt to determine whether altering the physical design of nests results in an altered pattern of colony activity.

Chapter 8 concludes this thesis, summarizing the findings of the experimental chapters. I also review other approaches to understanding cyclicity in colony activity that were not formally tested in the body of the thesis. I consider possible selective advantages to the pattern of activity observed. I present two models concerning synchrony, one couched from the perspective of the individual and the other in terms of efficient colony function. I further discuss aspects of model design and data interpretation, and briefly review potential avenues for further research in the field of activity patterns in ant colonies.

1.3 Self Organization and Mass Action

During the last decade, a number of researchers have considered social insect behaviour under the guiding principle *individuals operating simple decision rules lead to (occas ionally) complex and (presumably) adaptive patterns of behaviour at the colony level.* Hence, colonies exhibit emergent properties of a functional nature as a result of interactions between simple reducible units.

Related to the development of this approach have been studies of complex systems in other areas of science and mathematics. One such is the realization that the coupling of three or more simple nonlinear first order differential equations often results in complex outcomes that are ultimately deterministic but may be so dependent on initial conditions they are essentially unpredictable (i.e., chaotic: Li and Yorke, 1975; May 1976; reviewed by Eubank and Farmer, 1990). This has lead to the suggestion that populations of organisms, predator-prey and host-parasite systems may be similarly complex in system behaviour whilst determined by simple rules (May, 1976; Anderson and May, 1979; May and Anderson, 1979). Similarly, complex patterns such as leaf and plant physical structure (Prusinkiewiecz, 1991), structure in ecological systems (reviewed by Sugihara and May, 1990), and pattern formation in development (Meinhardt, 1982) may result from simple rules of growth and differentiation governing cell behaviour.

The concept of self organization was introduced by Nicolis and Prigogine (1977), with particular reference to chemical systems. Spatial and temporal patterns have been investigated in such systems, such as chemical oscillators (reviewed in Nicolis, 1989; Epstein, 1990;) and pattern formation in Belousov-Zhabotinsky reactions (Belousov, 1958; Zhabotinsky, 1964). Similar spatial patterns to those found in B-Z reactions are observed in cultures of the social amoeba *Dictyostelum*

discoideum (Shaffer, 1962; Gerisch, 1968), as a result of cell behavioural reactions to temporal oscillations in cAMP production (Malchow et al., 1978; Gerisch and Malchow, 1976).

The simple behaviours of neurons, when coupled together may yield more complex (emergent) outcomes (see for instance, Fentress, 1976). It has even been suggested that self organization through a process akin to natural selection may produce high level patterns of organization in the brain ("neural darwinism": Edelman, 1989). More generally, the discipline known as "neural networks" has developed in which networks of coupled units known as neurons are simulated by computer models, and evolve through repeated trial and error patterns of connection that yield outcomes designed by the modeller (reviewed by Cowan and Sharp, 1988; Rumelhart and McClelland, 1986). Although illustrating that simple units can produce complicated outcomes when coupled together (Hopfield, 1982; Kauffman, 1984), it is unclear whether the neural networks of computer science are realistic models of biological neural systems, and whether such simulations can yield testable hypotheses concerning neural organization (see for instance Crick, 1989).

Ants have been likened to cells undergoing differentiation during organismal development (Wilson, 1985a), and also to neurons in the brain (Minsky, 1987; Markl, 1985, Hofstadter, 1980:350). Interactions between ants are likened to connections between neurons; colony level behaviour patterns being the result of complex patterns of interaction, although the rules of interaction might be relatively simple. This type of reasoning led Wilson and Hölldobler (1988) to conclude that colonies are "dense heterarchies"; communication occurs between many units (ants) that are not arranged as a simple hierarchy and to a large extent do not possess any central control elements. Aspects of control and de-

centralization will be considered in the next section.

Studies of self organization in ants have mainly concentrated on foraging problems, owing to the difficulty of observation within nests. Recruitment to food sources via trail laying (reviewed by Hölldobler and Wilson, 1990: 265-279) has been studied, with particular reference to the choice of paths between food source and nest. Simulation models have been used to show that trail laying species can navigate efficient routes to food sources (Goss et al., 1990; Stickland et al., 1992) by virtue of individuals choosing to take routes in proportion to the ratio of strengths of trail pheromone they detect on various routes. Hence, individuals returning from a successful forage influence the behaviour of following ants; shorter paths obtain a stronger pheromone signal since they are more quickly traversed, so a higher number of successful foragers can return on them in a given time (for example Goss et al., 1990; see also Wilson and Hölldobler, 1988; Tofts, 1991). Using similar techniques, Deneubourg et al. (1989) have shown how species specific raid patterns of army ants may be self organized, with species differences relating to prey distribution. Empirical studies lend qualitative support to these models (Pasteels et al., 1987; Deneubourg et al., 1990; Franks et al., 1991a; de Biseau et al., 1992), although rigerous analysis of the effects of parameters such as forager caste size, rate of exit from the nest, path length, strength of influence of returning ants and pheromone decay rate lead to a variety of predicted outcomes (Stickland et al., 1992; Beckers et al., 1989).

A number of interesting features arise from these considerations. Since such systems are based on positive feedback (stronger signals elicit a stronger following response, so larger numbers then contribute to the signal), colony level responses (for example, recruitment to new food sources, "selection"of shorter paths) can occur quite rapidly.

Another feature of "mass communication" systems (Wilson, 1962) is that they are tolerant to faults (Oster and Wilson, 1978:11; Herbers, 1981b). Individual units can make mistakes and be of relatively low quality without failure to operate effectively at the system level. "Mistake" making is illustrated by the probabilistic nature of path choice in models (Stickland et al., 1992; Goss et al., 1990), and observations of individuals taking inefficient paths or abandoning the trail in experiments (de Biseau et al., 1992; Pasteels et al., 1987). Pasteels et al. (1983) have suggested that some level of mistake making or "noise" is adaptive since it allows new food sources to be located, and more efficient routes to be tested.

These studies suggest that when systems contain large numbers of units, the required patterns of behaviour can emerge from individuals operating simple rules. Consequently individual units need not be capable of complex behaviours, and may thus be less costly to produce. In the situations discussed above, individual ants need not navigate on the basis of external cues such as landmarks, and need not employ compasses for navigation. Hence the evolution or maintenance of sensory and processing systems required for landmark recognition or direction finding becomes unneccessary. Equally, the fault tolerant nature of these systems allows individual components (ants) to be lost (for example due to navigation errors or predation) without excessive harm befalling the colony. Hence the production of many, simple units allows fast and flexible response to environmental contingencies, whilst mass communication and self organization leads to redundancy of individuals such that their loss or imperfect function can be tolerated (Herbers, 1981b; Oster and Wilson, 1978:14).

Other aspects of behavioural control in ants have also been studied using the self organization approach. Pasteels et al. (1987) and Goss et al. (1990) have shown using simulation models how foragers may recruit to food sources of higher

quality, if the strength of trail laying is related to food quality (calorific value, or protein content). These models are supported by empirical observations on proportions of foragers laying trails (de Biseau et al., 1992) for different food sources. Franks (1986) has shown that army ants (*Eciton burchelli*) appear to form groups (or "teams") for retrieval of larger prey items by a process of self organization. The rule employed appears to be that individuals join a group and help carry the prey if it is moving at a rate slower than other items in the foraging stream.

Intranidal behaviour may also be organized on the basis of simple rules. Among the most important problems that ants within the nest must "solve" is that of task allocation, or what job an individual should do. Wilson (1985b) demonstrated that task allocation in *Pheidole pubiventris*, which possesses physically distinct castes, may be flexibly regulated in a simple manner. Minors normally carry out brood care, but if they are removed majors (normally specializing in defence) take over this task. It appears that majors allocate themselves on the basis of a rule *avoid minors in the presence of brood.* Hence when minors are no longer present, aversive behaviour no longer occurs and majors reallocate to the brood pile. Similarly, differing rules governing the behaviour of majors and minors in response to colony attack may lead to flexible alternative defensive strategies in *Pheidole pallidula* (Detrain and Pasteels, 1992).

Behavioural dynamics of task allocation outside the nest have also been studied by Gordon (1986, 1987) for the harvester ant *Pogonomyrmex barbatus.* These studies indicate that complex shifts between tasks such as nest maintenance, foraging and patrolling occur when the colony is subjected to environmental manipulations. Gordon et al. (1992) have simulated these changes using a neural network model of colonies, where individuals change state (task or activity level)

on the basis of inputs from other individuals. Finally, patterns of brood arrangement within nests of *Leptothorax unifasciatus* have been studied by Franks and Sendova-Franks (1992). It is likely that self organization principles account for these patterns, although the precise sorting rules operated by individuals remain unclear at present (Deneubourg et al., 1991).

A number of interesting studies of honeybees (*Apis mellifera*) point to self organization as a mechanism for worker allocation, foraging strategy and spatial pattern formation. Seeley (1989a) has demonstrated that foragers may regulate their foraging rate on the basis of queuing times to offload nectar on return to the nest (see also Section 8.3.1). Similarly, selection between nectar sources may occur as a result of differing recruitment rates to sources of different profitability. Foragers appear to assess source quality independently; the frequency of dance circuits performed on return to the nest reflects this assessment and consequently enhances or decreases recruitment rate to the source (Seeley et al., 1991). Within hives, spatial patterns of brood, pollen and honey appear to be self organized as a result of dynamic interactions between rates of brood, honey and nectar placement in cells (Camazine, 1991). If the self organization principle operates in these situations, then individual workers produce patterns in time and space (which may be adaptive) as the outcome of local interactions; individuals themselves do not possess global knowledge of the patterns.

1.4 The Nature of Interactions

By definition (Michener, 1974; Wilson, 1971:4; Hölldobler and Wilson, 1990:638), eusocial insects exhibit reproductive division of labour. Further, almost all ant species studied to date exhibit another division of labour, whereby individuals

specialize in a subset of the colony tasks not directly related to reproduction (for a possible exception see Traniello, 1978). Individuals may specialize in a single subset throughout adult life, as is the case for physically distinct castes (reviewed in Hölldobler and Wilson, 1990:320) or they move through a number of subsets; the direction and timing of these changes appears to be associated with age (see Hölldobler and Wilson, 1990:320; Calabi, 1988).

Specialization may enhance the efficiency of colony operation for a number of reasons. Firstly, individuals may be physically specialized to perform certain tasks and thereby able to perform them more efficiently (for example; seed millers, *Solenopsis germinata* Wilson, 1978; defence workers, Hölldobler and Wilson, 1990:330). Alternatively, prolonged repetition of tasks may enable some individuals to learn to perform them more accurately (Hölldobler and Wilson, 1990:365). Perhaps of greatest importance is that division of labour entails division of problems: tasks at the colony level, such as raising brood, are divided into a sequence of steps performed by individuals, such as cleaning and feeding brood, and foraging.

Further, these operations are performed concurrently (for instance, many brood items are raised at the same time). Oster and Wilson (1978:12) describe this system as operating in series-parallel. The system is thought to be more reliable than one that operates in series (Oster and Wilson, 1978:14; Herbers, 1981b), since failure of one element to complete a step does not lead to breakdown of the task network, as other elements may take over that step (compare the reliability of Christmas lights wired in series or in parallel). The concurrent system thought to operate in social insects is analogous to a conveyer belt production line, in which a number of workers are assigned to each processing stage. Unlike human production lines however, there are not thought to be any central control

elements (supervisors) that direct workers to take up positions to maximize production rates (Wilson and Hölldobler, 1988; but see Reeve and Gamboa, 1983). Workers may allocate themselves appropriately by simple (self organizational) principles, in response to shortfall or surplus that they perceive locally (Tofts, 1991a, 1991b). An important feature of such systems from the perspective of information exchange is that workers may be expected to respond to local cues in order to "decide" which tasks it is appropriate to perform. Hence we might expect interfaces between task sites to be important locations of information exchange, concerning task allocation or rate of task performance. Equally, if ants perform tasks in series (there is no division of labour), we might expect the occurence of such information exchange to be reduced (as discussed in Chapter 2).

1.5 Patterns of Activity in Ant Nests

Although considerable research has concentrated on what individuals do (see previous sections), relatively little attention has focused on when individuals do it. Herbers (1981a) in a theoretical analysis of satisfaction models of foraging concluded that for many organisms it is advantageous to be inactive much of the time. Although she did not consider time constraints on other behaviours, her point that inactivity itself should be considered as a trait subject to natural selection is worth considering. Observers have for some time noted that many organisms, particularly invertebrates, spend considerable portions of their time inactive (Elton, 1927). Unfortunately, many studies of behavioural repertoires of social insects have not considered inactivity as a behavioural act (for example Wilson and Fagen, 1974). Those that have generally conclude that on average, individuals spend up to three quarters of their time quiescent (Herbers and Cunningham, 1983; Herbers, 1983; Cole, 1986; Allies, 1984).

Such levels of inactivity perhaps indicate that the work force in social insect colonies greatly exceeds the work required under normal circumstances. This reserve might be advantageous since it can be mobilized to deal with threats to the colony, such as predator invasion or nest destruction (Hölldobler and Wilson, 1990:342; Michener, 1964). When adverse conditions do not present themselves, it follows that the reserve work force should reduce energy expenditure by remaining inactive, if colony net productivity is to be maximized. Conflicts of interest between individuals (for instance over male production or relatedness to brood) may also yield differing patterns of activity between individuals, or between populations employing different reproductive strategies (Schmid-Hempel, 1990). Although it is possible for such a system to be organized as a set of individuals that always engage in work if it is available and another set that remains inactive until an emergency, this does not appear to be the case. Many studies suggest that individuals differ in the average proportion of time spent inactive (Herbers and Cunningham, 1983; Herbers 1983; Cole, 1986), but there is no evidence for an inactive caste *per se*.

Given, then, that individuals all cycle through states of activity and inactivity, one question of interest is how the schedule of state changes is determined. Recently, observations of laboratory colonies of leptothoracine ants have suggested that activity within nests is synchronized, such that individuals are generally active (or inactive) together. This phenomenon was first reported by Franks and Bryant (1987), studying video recordings of *L. acervorum* nests. Sychronization tended to result in colony level rhythms of activity of the order of 20 minutes (see also Franks et al., 1990a). Cole (1991a) reports similar activity rhythms in *L. allardycei*, with an average period of 26 minutes. The precise methods used in these studies are discussed in detail in Chapters 3 and 8.

The original study (Franks and Bryant, 1987) of this phenomenon involved data collected manually. The state of individuals (active or inactive) was recorded at 1 minute intervals from video recordings of the colony; the recording was replayed to obtain simultaneous data for many individuals. This technique entailed a large investment in data collection for relatively low yields of information; time series for 4 colonies were obtained, each of 10 hours duration, based on observations of 20 workers (plus queens) throughout that period.

The difficulty of collecting sufficient quantity (and quality, in terms of sample size per colony) of data has led to conflicting conclusions as to the pattern of activity in colonies. Franks and Bryant (1987) describe it as "rhythmical", although Franks et al. (1990a), using manual and automated techniques, conclude that it is "nonperiodic", but that activity is synchronized and occurs in short "bursts" or "pulses". Cole (1991a), using automated techniques, describes the pattern as both "rhythmic" and "periodic". Further, lack of sufficient quantities of data has precluded the testing of models (Goss and Deneubourg, 1988; Hemerik et al., 1989; Tofts, 1990a) of the mechanism underlying the pattern. These models are discussed in detail in Chapter 4.

1.6 Leptothorax acervorum: behaviour and ecology

1.6.1 Evolution

Wilson (1971:4) defines eusociality as the possession of the following features: (1) a number of adults living within the same nest; (2) cooperation between adults in brood care and nest building; (3) reproductive division of labour such that

some individuals are reproductively dominant or sole reproductives; (4) overlap of generations such that offspring may care for siblings.

True eusociality occurs in the Isoptera, Hymenoptera, and arguably the Mole-rat (Sherman et al., 1991). Within the Hymenoptera, all species of ants (Formicidae) are eusocial, and are thought to have derived from a single common vespine ancestor in the Cretaceous (Wilson et al., 1967; Wilson, 1987). Hymenopteran systematics has been reviewed by Evans (1958), Brothers (1974), Snelling (1981) and Brockmann (1984). The Formicidae have been divided into two complexes (Brown, 1954; Wilson, 1971), or more recently into 11 (extant) subfamilies (for example, Hölldobler and Wilson, 1990:26).

Within the present scheme, the genus *Leptothorax* is contained within the subfamily Myrmicinae, a relatively evolutionarily "advanced" subfamily (containing many derived as opposed to ancestral character states). The Myrmicinae represent the most specious subfamily, containing such diverse species as the granivorous Harvester ants (*Pogonomrymex*), fungus cultivating Attines, and predatory Datecines. The subfamily is represented worldwide (apart from Antartica); species range in mature colony size from less than 100 individuals (some leptothoracines) to over a million (as in *Atta*; Hölldobler and Wilson, 1990: 162). Workers in species range from apparently monomorphic (Herbers, 1983) to two or more physically distinct castes associated with a division of labour (for instance, *Pheidole*: Wilson, 1976). Although the other subfamilies have been divided into robust tribes, the taxanomic relationships among members of the Myrmicinae are still poorly understood (Snelling, 1981; Hölldobler and Wilson, 1990:16), and the situation at the level of genus *Leptothorax* is still similarly confused. Leptothoracine classification is considered by Bolton and Collingwood (1975), Collingwood (1979), Buschinger (1987) and Douwes and Stille (1987).

Leptothoracines have been divided into two complexes; *Myrafant* and *Leptothorax sensu stricta* (Buschinger, 1987; Douwes and Stille, 1987), over forty species occuring in Europe (Collingwood, 1979:68). The former grouping includes among others *L. unifasciatus*, *L. tubero-interruptus*, and *L. allardycei*; each small in colony size, with one queen per nest, which is usually morphologically quite dissimilar from the workers. *L. acervorum* is contained within the *Leptothorax s.s.* grouping, and also exhibits small colony size, although workers and queens are of similar size and appearance, and nests frequently contain more than one queen (Heinze and Buschinger, 1988).

Leptothoracines have largely been studied owing to their small mature colony size (up to 300 individuals; Collingwood, 1979:68), and ease of culture in the laboratory. Colonies can be housed in nests (glass tubes or flatter, rectangular structures; for examples see Herbers and Cunningham, 1983; Bourke, 1991), that allow observations of intranidal behaviour. Owing to this suitability, leptothoracines have been studied in the context of behaviours within the nest, such as division of labour (Herbers, 1983; Herbers and Cunningham, 1983), dominance interactions (Cole, 1981; Franks and Scovell, 1983), and aspects concerning propensity towards polygyny (Buschinger, 1968a; Heinze and Buschinger, 1988), and social parasitism (for example Wilson, 1975; Buschinger, 1990; Bourke and Franks, 1991). These aspects are considered below.

1.6.2 Ecology

Leptothoracines are widespread in Europe, Asia, the United States and Canada. *L. acervorum* occurs in temperate regions of Europe and North East Asia; the key of Bolton and Collingwood (1975) provides features for identification and

some notes as to range. Mature *L. acervorum* colonies contain up to 200 or so individuals (pers. obs.; P. Douwes, pers comm.), and inhabit nests in fallen sticks, tree stumps, bark, rotten or buried branches, under peat or stones (Collingwood, 1979:72; Bolton and Collingwood, 1975:19) or in cavities between rocks (N. R. Franks, pers. comm.). Internal structure of nests in the field is dependent upon physical structure of the nest medium: nests in branches may consist of a ramifying network of chambers, containing brood at a variety of developmental stages (pers. obs., see also le Masne, 1953 for other leptothoracines), nests in rock cavities may contain a single central brood pile, although brood is usually limited to a small number of major chambers (P. Douwes, pers. comm.). In laboratory nests, the brood is usually clustered in a single pile towards the centre of the cavity, often at the back of the nest away from the entrance (pers obs., N.R. Franks, A.B. Sendova-Franks, pers comm). Brood items are separated by some distance, allowing workers access to the brood, although eggs and smaller items are often clumped together (Franks and Sendova-Franks, 1992; le Masne, 1953).

Evidence on food sources for this species is limited: Bolton and Collingwood (1975:19) suggest this species is mainly a scavenger although it also preys on small insects and has not been observed to tend aphids. In the laboratory they can be successfully cultured on Bhaktar Whitcomb diet (Bhaktar and Whitcomb, 1970), honey or sugar water, and protein in the form of live *Drosophila* larvae or dead locusts (A. Bourke, pers. comm.). Foragers are not thought to lay trails to or from food sources, rather foragers often act independently, but may recruit to food sources or move to new nest sites using a mechanism known as tandem running (Wilson, 1971:248; Möglich et al., 1974; Möglich and Hölldobler, 1974). Here, the successful forager returns to the nest and leads other workers to the food source by direct physical contact between the leader's abdomen and follower's antennae. The leader may release pheromone at the nest site as an

invitation to recruits (a mechanism known as tandem calling: Moglich, 1979).

Colonies of *L. acervorum* are founded independently by single queens after a nuptial flight (Buschinger, 1971a), although measurements of genetic relatedness between queens and polygynous colonies suggest that daughter reproductives may be adopted by mother nests (Stille et al., 1991). However, the observation of a nuptial flight in this species, in which large numbers of reproductives gathered in a mating cloud some distance from suitable habitat for colony maintenance (Franks et al., 1991b), would suggest that daughter adoption is not the sole strategy employed in this species. *L. acervorum* queens release a sexual calling pheromone to attract mates (Franks et al., 1991b), so it is possible that some queens mate close to the mother nest, and are not involved in a nuptial flight. There is also some evidence for adoption of (presumably alien) mated females by *L. acervorum* nests (Stille and Stille, 1992). There have been no observations of mating from nests confined to the laboratory (Buschinger, pers. comm.; Bourke, pers. comm.). Typically, female sexual brood overwinter twice, and those of males and workers once (Buschinger, 1973). The overwintering period and suitably variable temperature regimes appear to be necessary for successful development to pupation in laboratory colonies (Buschinger, 1973). Males develop from unfertilized eggs (haplodiploidy); leptothoracine workers may contribute to the colony production of males through direct egg laying (reviewed by Choe, 1988; Bourke, 1988a).

Studies of division of labour in leptothoracines have revealed the existence of behavioural castes in the species *L. longispinosus* (Herbers and Cunningham, 1983). Although workers are not physically distinct, size appears to be related to labour division. It is generally assumed that some form of age related division of labour (see Section 2.1.3) occurs in Leptothoracines (Herbers, 1983; Cole, 1986, 1992). There does not appear to be direct experimental evidence for division of

labour in *L. acervorum*; in Chapter 2 I attempt a minimal investigation of this question.

Wilson and Fagen (1974) estimate the behavioural repertoire of *L. curvisponsus* to contain 29 different behavioural acts. The problem of act definition (Section 2.1.2) is illustrated by comparison to Herbers and Cunningham (1983), who report 37 acts for *L. longispinosus* and Herbers (1983), reporting 46 acts for *L. ambiguus*. Herbers (1983) and Herbers and Cunningham (1983) consider that the repertoires conform closely between species. Herbers and Cunningham (1983) group behavioural acts into four roles: personal behaviour, brood care, social interactions and colony maintenance. Acts and roles are considered further in Section 2.2.

Other behaviours noted in leptothoracine workers include laying of trophic and male-producing eggs (Cole, 1986; Bourke, 1988b), dominance interactions (Cole, 1981; Franks and Scovell, 1983; Bourke, 1988b; Heinze and Smith, 1990), and sorting of brood such that smaller items are gathered in the centre of the brood pile, and later instars and pupae dispersed around the periphery (Franks and Sendova-Franks, 1992). Leptothoracines also appear to possess simple building behaviours, such that sand grains and other items are moved into positions around the brood pile as a loose wall (Franks et al., 1992). Most relevant to this thesis, leptothoracine workers appear to synchronize their activity, such that colony level pulses in activity occur, with a period of roughly 20 minutes (Franks and Bryant, 1987). Indirect evidence (Franks et al., 1990a; Cole, 1991b) suggests that synchronization may result from a propensity of inactive individuals to respond to physical contact from active individuals. This feature is investigated further in Chapter 5.

1.6.3 Polygyny

Hamiltonian arguments would suggest that both reproductives and sterile workers in a colony should favour monogyny (that is, a single laying queen per colony). Queens would maximize their inclusive fitness as sole reproductives, since they are more closely related to their own offspring than to those of other relatives or non-relatives. Similarly, workers should elect to raise sisters rather than half sisters or even less closely related brood. However, in many species of Formicidae, polygyny occurs; nests may contain from a few (for example, some leptothoracines; Buschinger, 1968a; Bourke, 1991; Heinze and Buschinger, 1988), to many thousands of individuals that are queen like in morphology and function (for example, *Monomorium*; and other "tramp" species: Hölldobler and Wilson, 1990:215).

L. acervorum is facultatively polygynous; colonies may possess more than one functional queen, although mongynous colonies also occur. This species demonstrates the difficulty in defining levels of gyny: colonies can contain one or more fertile, egg laying queens, plus one or more unfertilized young or old individuals that are queen like in morphology but do not lay eggs, and also one or more young, fertilized females of queen like morphology that are yet to lay eggs (Buschinger, 1968a, 1978). In Japan, *L. acervorum* (or a closely related species: N. R. Franks, pers. comm.) appears to be functionally monogynous; although roughly half the colonies studied contained more than one individual of queen like appearance, generally only one individual per colony was an inseminated egg layer (Ito, 1990). Genetic studies of relatedness between workers in *L. acervorum* colonies from Sweden reveal that a direct count of fertile queens in polygynous colonies may overestimate effective queen number, whilst underestimating it in apparently monogynous colonies (Stille et al., 1991). This suggests that presently monogynous colonies may previously have contained more functional queens, and

that in polygynous colonies, some form of dominance operates such that some queens are more productive than others. The mechanism whereby this dominance operates is unclear; Bourke (1991) found no evidence of antagonistic behaviour between queens, or selective killing of another's offspring.

A number of hypotheses concerning the adaptive advantage of polygyny have been suggested. One such relevant to *L. acervorum* i s the risky nature of queen and colony survival in species with small colonies inhabiting limited and vulnerable nest sites. When nests are contained in cracks in bark or rocks, or hollows within sticks, portions of the nest might be expected to be lost or dispersed quite frequently, due to trampling by larger animals, flood and wind. In such circumstances, it might be advantageous for related queens to associate, such that individual portions retain an egg layer when they become split (Hölldobler and Wilson, 1977, 1990:213). Herbers (1986a,b) has demonstrated a positive relationship between degree of secondary polygyny and colony density in *L. longispinosus* (inhabiting acorns), suggesting a link between polygyny and nest site availability. Heinze (1992) notes that for leptothoracines, functional monogyny is associated with species inhabiting isolated or patchily distributed habitats, and that polygynous species occur in more widespread homogenous environments, and suggest that polygyny may hence be related to a reduced need for long distance dispersal, and an increased reliance on dispersal through colony budding.

Associations of nonrelatives in nest foundation may be advantageous if colony foundation is particularly costly or risky, such that the costs of altruism towards nonrelatives is outweighed by the benefits of association, such as reduced work on nest excavation, or more rapid initial growth of the workforce (Evans, 1958, 1977; West Eberhard, 1981).

1.6.4 Social parasitism

The propensity of *L. acervorum* colonies to accept multiple queens may have resulted in increased vulnerability to social parasitism in this species.

Social parasitism occurs in five of the subfamilies of the Formicidae; especially the Myrmicinae and Formicinae. It can take one of three basic forms, although intergradations occur; temporary parasitism, inquilinism and dulosis. The only basic type not exhibited by leptothoacines is temporary parasitism, in which the parasite queen invades the host colony after nuptial flight, kills the host queen and proceeds to lay eggs cared for by the host workers. This form is exhibited within the genus *Formica* (reviewed in Hölldobler and Wilson, 1990:444).

Among leptothoracines and the closely related genera *Doronomyrmex*, *Epimyrma*, *Formicoxenus* and *Harpagoxenus*, 24 parasitic species are known (Hölldobler and Wilson, 1990: 439), each associated with one or more closely related species.*L. acervorum* itself is a host of 5 parasitic species, and in common with other such host-parasite arrays, the geographical range of the host is much greater than that of any of its parasites. *L. acervorum* social parasites have not been found in Britain, and appear to be extremely localized within the continental range; occuring in isolated arreas of Austria, Southern France, Northern Italy, Germany and Sweden (Hölldobler and Wilson, 1990:438; Franks, pers. comm.). As with the North American parasites of widespread Myrofant hosts *L. ambiguus*, *L. curvispinosus* and *L. longispinosus*, parasite species are localized but often occur sympatrically (Buschinger and Alloway, 1979).

Inquiline species do not kill the host queen, thereby ensuring continued production of host "slaves", and maintenance of the host species. Inquiline species

complete their lifecycles within the host nest, often mating close to or within it. Such a relationship is found between *Doronomyrmex kutteri* (inquiline) and *L. acervorum* (host) (Buschinger, 1965). Inquilines appear to override the host species' colony recognition cues, by release of a "propaganda substance"(Allies et al., 1986), and by grooming the host queen to allow disguise (Franks et al., 1990b). The inquiline lays eggs cared for by the workers, and which develop into reproductives; in this species and others a worker caste is largely unknown (Buschinger, 1965; but see Buschinger, 1982). The species *D. goesswaldi, L. buschingeri* and *D. pacis* are also inquilines of *L. acervorum* (Buschinger, 1974a, 1990).

Dulotic species (slave raiders) exhibit a well developed worker caste, which is employed to raid host colonies and retrieve brood, which act as a slave work force in the parasite's nest on eclosion. *Harpagoxenus sublaevis* is a slave raider of *L. acervorum* (Buschinger, 1968b); raids are performed on neighbouring host colonies in summer, wherein they retrieve host pupae and prepupae (Buschinger, 1983). This species also appears to employ "propaganda substances" that confuse the host's colony identity, such that host workers have been observed to attack each other during and after raids (Buschinger, 1974b; Allies et al., 1986).

Both dulotics and inquilines appear to be close taxonomic relatives of their hosts (Wilson, 1971:360; Buschinger and Alloway, 1979; Buschinger, 1965). Buschinger (1965, 1970, 1990) has suggested that such relationships might be explained by sympatric speciation of the parasite from the host species (see also Bourke and Franks, 1987, 1991). Under this mechanism, inquilines may evolve if a mutant population of queens that are unable to produce a worker caste remain tolerated by incipient hosts, but become reproductively isolated from them. The occurrence of intermediate forms between microgyne (inquiline) and macrogyne (host)

in certain *Myrmica* species (Elmes, 1978; Pearson, 1981) lends support to this hypothesis. An alternative explanation is that inquilinism represents degeneration of dulosis, and that dulosis itself evolved from territorial conflicts (Wilson, 1971:364, 1975; Alloway, 1979, 1980) or predatory relationships (Darwin, 1859) between incipient host and parasite. The proposed mechanism entails that predators or competitors, capturing brood for food or competitor interference, may be selected to retain brood to eclosion, thereby gaining a slave labour force. Under this mechanism, parasites speciate allopatrically, since they do not derive from the host species. Buschinger and Winter (1982, 1983) provide evidence that the evolutionary trend in the parasite array *Epimyrma* is from dulosis to inquilinism, although it is still possible to account for dulosis through sympatric speciation (Buschinger, 1970).

In summary, it seems likely that certain features of the ecology and reproductive strategies of *L. acervorum* predispose this species to social parasitism. These features may include tolerance to multiple queens, polydomy and possible exchange of work force between nest sites, limited or ephemeral nest sites, and intercolonial territoriality and aggression.

Chapter 2

Information Exchange Within Ant Nests

2.1 Information Exchange

Central to the theme of information processing in social insects is the concept of communication. Functions and mechanisms of communication in ants take a variety of forms, many in common with other animals (singular or social) and some unique to eusocial species. From the viewpoint of this thesis, it is necessary to ask what information should be exchanged between colony members, to allow consideration of potential links of information processing and activity patterns. I shall attempt to investigate this question by conducting experimental observations of laboratory based colonies.

2.1.1 Communication and Social Organization

Direct communication between individual ants can broadly be assigned to two categories of mechanism: chemical and tactile. Wilson (1962) recognized the im-

portance of chemical communication in ants, and Hölldobler and Wilson (1990: 229, 263, 266) review the vast array of chemical signals known to operate in ant species. Although all communication is in essence mediated at the level of individual senders and recipients, (Krebs and Dawkins 1984), the effects of such communication in social insects can also be the basis for organization at the colony level. Chemical signal have been refered to as pheromones (Karlson and Lüscher, 1959; Norlund, 1981) irrespective of their physiological origin or function. Norlund (1981) also considers semiochemicals from an adaptive perspective, distinguishing allomone (interspecific semiochemical, adaptively favourable to the emitter) and kairomone (interspecific chemical, favourable to the receiver) (see also Brown et al., 1970a). For our purposes, the variety of chemical and tactile methods of communication are perhaps better approached from a functional perspective.

Communication may be the result of one to one interactions, by direct interaction between individuals, or one to many, whereby diffuse chemical or vibrational signals released by one individual can be perceived and reacted upon by many. We can consider signals as falling into one of three broad categories: (1) those related to recognition of identity (which I shall refer to as labels); (2) those involved in mediating direct interactions between individuals (immediate signals) and; (3) those involved in changing the long term behaviour of individuals (modulators).

Labels are involved in species recognition, colony recognition and in some cases caste recognition. Queens of *L. acervorum* release sexual calling pheronomes after nuptial flight that attract potential mates of the correct species (Buschinger, 1971a; Franks et al., 1991b). Species and colony specific subcutaeneous chemical components, refered to as colony odours are thought to be involved in species and colony recognition by adults (Hölldobler and Wilson 1990: 203). Hölldobler and

Wilson (1990: 200) suggest that similar mechanisms allow identification of castes within colonies; queen and brood in many species possess 'caste specific' odours that are attractive to workers and may elicit grooming and feeding behaviours and also there is evidence of individual recognition odours (Maschwitz et al., 1986). Dead nestmates appear to be recognised by the possesion of oliates and oleic acid, which stimulate disposal behaviours in workers (Wilson et al., 1958; Blum, 1970).

Immediate signals are generally involved in daily colony maintenance rather than colony recognition and cohesion. Colony maintenance entails recruitment to foraging sources, or large scale reactions to colony threatening occurences, energy distribution, and some aspects of worker organization within the colony. These signals include active pheromone release from various glands, most commonly Dufour's gland, the poison gland or sternal glands in trail laying species (reviewed by Hölldobler and Wilson, 1990: 266), or active secretion from the poison gland in *L. acervorum* (Möglich et al., 1974) in tandem calling behaviour; both elicit an immediate "follow me" response, utilized for recruitment in foraging or emigration activities. Recruitment and alarm often involve positive feedback ,whereby the receiver of a signal also emits the signal as part of their reaction to it, thus leading to mass action phenomena causing gross changes in the colony behaviour as a whole (for example, absconding in *Pheidole*, Wilson 1976b; reinforcement of trails, Franks et al., 1991a).

Various physical systems of communication also serve as immediate signals in ants. Many species exhibit body drumming against the substratum, or stridulation (the rubbing together of specialised body parts) as acoustic signals for alarm or recruitment (Markl, 1983, 1985; Markl and Hölldobler, 1978). Stridulation is also employed as a courtship terminator and as a modifier of other behavioural

responses (Markl and Hölldobler, 1978). Tactile communication in the form of antennation is important in initiation and maintenance of tandem running recruitment and solicitation of food exchange by trophallaxis (Hölldobler and Wilson, 1990: 293). Besides serving a nutritive function, trophallaxis may be involved in the organization or control of patterns of dominance between individuals. Cole (1981), reports linear dominance hierarchies among laying workers of *L. allardycei*, as do Franks and Scovell (1983) for *H. sublaevis*. Franks and Scovell report that the queen obtains more frequent trophallaxis from dominant individuals, thereby possibly limiting energy that could be utilized for egg production.

Another tactile interaction leading to immediate response reported in *S. invicta* (Hölldobler and Wilson, 1990: 228) and *L. acervorum* (pers. obs.; Cole, 1991b) is casual antennal or bodily contact between workers. It can be considered as a form of communication in that it elicits increased undirected movement or turning towards the sender. Such casual contacts could serve a colony cohesion function, or if purely stimulating movement activity, the response to their occurence may be linked to maintaining the colony in a state of readiness to respond, for instance to catastrophes such as invasion by a predator, or nest destruction (see Chapter 8). I shall argue in this thesis that such casual contacts appear to be responsible for short term pulsatile activity in colonies, by a process of positive feedback, and that such interactions may lead to a variety of potential organizational benefits to the colony.

Behavioural modulators are involved in what can be loosely termed colony "development". Queens of *S. invicta* produce a pheromone that inhibits dealation of virgin queens (Fletcher and Blum, 1981). Some species with physically distinct worker castes appear to utilize chemical methods of caste determination: *Pheidole* majors produce a pheromone that inhibits major development by de-

sensitizing pupae to juvenile hormone (Wheeler and Nijhout, 1981, 1983, 1984). In some polygynous species, dominant queens appear to inhibit ovary development of other queens, and in many species it is thought that queens inhibit worker egg laying by similar mechanisms (Passera, 1980; Hölldobler and Wilson, 1983; Buschinger, 1979).

The precise nature of communication within social insect societies is frequently linked to the particular form of social organization therein: broadly speaking, colony function depends upon appropriate intra and intercaste communication. Hence we need to consider the existence and nature of caste within ant colonies.

2.1.2 The Concept of Caste

Wilson (1953, 1971:136) defined castes as sets of individuals differing morphologically and with specialized functions; Michener (1974), refering to castes in bees, described them as physiologically, behaviourally, and sometimes morphologically different forms, but excluded age groups from the definition. Wilson (1968) broadened his definition of caste to include age groups with specialized behaviour; this definition is further broadened by Oster and Wilson (1978:19) to be of a purely functional nature; castes were defined as sets of individuals that perform specialized labour for 'sustained periods of time'. This broad definition is retained by Hölldobler and Wilson (1990:300).

Buschinger (1978) argues Michener's (1974) functional definition of caste is preferable to Wilson's (1971) morphological definition since form and function do not neccessarily correspond. His argument is couched with reference to queen polymorphism in ants (see Chapter 1). Peeters (1990) prefers a morphological defi-

nition of caste, suggesting the term role for behavioural subsets of workers. His argument is couched with reference to evolution of the reproductive division of labour. Although Peeters wishes to separate the dual meanings of caste with respect to reproductive and other divisions of labour, his definition does not clarify the situation, since physically distinct groups of workers are not reproductively distinct (Buschinger, 1978).

Within the context of this chapter, I am concerned with worker function. In general, I shall utilize Oster and Wilson's broad definition of caste. However, this definition perhaps over emphasizes the static nature of labour division within some ant species, especially those with no physically distinct worker castes. It is also difficult to determine what constitutes a sustained period of time from some experimental data (including my own), in which case I will refer to task groups.

Task group: a group of individuals that perform a subset of the colony repertoire of behavioural acts; these acts being linked to the performance of a particular task at the colony level. I define a task as: a set of behavioural acts which achieve some function for the colony (Sudd and Franks, 1987:66). Sudd and Franks (1987:66) define a behavioural act as "a logical unit like grooming, trophallaxis or carrying a larva". Unfortunately, there will inevitably be an element of subjectivity in the observer's choice of logical unit.

2.1.3 Caste: Explanations and Predictions

Oster and Wilson (1978:19) in common with other authors (Sudd, 1982, Calabi, 1988) observe that division of labour appears to occur in almost all ant species studied to date, such that castes exist by the broad definiton of Oster and Wilson

(1978:19). Physically distinct worker castes occur in a minority of species, and only 15% of genera (Hölldobler and Wilson 1990: 317). Surveys of the literature often do not reveal this owing to a historical bias in choice of study species when considering division of labour. Sudd and Franks (1987:79) point out that not a single discretely polymorphic ant species occurs in the British Isles.

The rarity of physically distinct worker castes requires explanation. Wilson (1968, 1971:341) modelling ant evolution with respect to optimizing work related efficiency at the colony level (colony ergonomics) concluded that in a constant environment and with no developmental constraints on worker morphology, the optimal solution is to produce one specialized worker caste per task. Oster and Wilson (1978:189) present a number of explanations for specialized caste rarity, including the developmental constraints of allometry (see also Franks and Norris, 1987); temporal constraints, that is, a time lag between the onset of new conditions and response time by the colony (i.e., the period of larval development); energetic constraints including the cost of maintenance of a feedback mechanism to determine size distribution, the cost of production of specialized forms that may rarely be required, and the need for behavioural flexibity at the individual level to cope with task overlap and environmental variance.

Another possibility is that the evolution of physically specialized castes is constrained at the level of individual. If inclusive fitness includes a contribution of male offspring, individuals with aberant physical morphologies incapable of male egg laying would suffer a decrease in inclusive fitness (Bourke 1988a; Sudd and Franks 1987:76).

The effective caste number can be increased without physical specialization by temporal or size related specialization. In the case of temporal polyethism, typi-

cally young workers tend to be associated with brood care tasks, and older workers are associated with foraging (Otto, 1958 in Sudd, 1982). West-Eberhard (1979, 1981) proposed that such age specific systems may have evolved as the result of mixed strategies of selfish reproduction followed by altruistic service after the last age of reproduction. Without considering the laying abilities of workers, selection may still favour the direction of task change, since older workers represent a disposable caste (Porter and Jorgensen, 1981) and are therefore most effectively deployed in the most dangerous tasks.

Studies of temporal polyethism suffer from the problem of accurate age determination of worker age by the observer. Methods (reviewed in Sudd, 1982) include aging by cuticular pigmentation, the extent of ovary development and mandibular wear. Arguably, these measurments may be related to task as well as (or instead of) age: mandibular wear may be related to extent of mandible use (exacerbated by tasks such as nest repair) and ovary development may be related to proximity to queen or brood.

Many studies have shown a correlation between either size or apparent age and task in species with no true polymorphism (and, indeed, within some castes of polymorphic species as well), as reviewed in Hölldobler and Wilson (1990: 320) and Calabi (1988).

These observations lead Hölldobler and Wilson (1990) to conclude that

"workers of almost all kinds of social insects change roles as they grow older" (Hölldobler and Wilson 1990:312).

Clearly, role cannot determine age (see Section 8.3.6). There has therefore been

a tendency to conclude that age determines role, by mechanisms such as developmental switches in genes involved in expression of substances that modulate behaviour.

In developing an ergonomic theory of caste, Oster and Wilson (1978:144) generalize this relationship by suggesting that workers undergo physiological change with age in such a way that responsiveness to environmental stimuli change.

They go on to state that the precise caste composition (caste distribution function: CDF; Oster and Wilson, 1978:157) of colonies may be the result of natural selection. That is, certain CDFs may be favoured to suit the environmental contingencies of particular populations and species. Such selection of caste ratios and numbers is refered to as "Adaptive Demography" (Oster and Wilson 1978:159; Hölldobler and Wilson 1990:307), since it implies (as caste is a consequence of age) that birth and death schemes have evolved to optimize caste ratios with respect to the species' environment. Experimental evidence in support of this hypothesis is lacking (Calabi and Traniello, 1989).

Calabi (1988) has argued that such tight coupling between age or size and behavioural class might not be adaptive, since it allows insufficient colony level response to short term environmental fluctuations. If age is the sole determinant of role, a colony would be unable to respond to the sudden loss of a caste until existing workers or new brood aged to the appropriate point. She argues that deviations of individuals from the expected age typical behaviour have been overlooked as noise in individual variability, whereas they may actually reflect true underlying flexibility that is signal rather than noise.

Calabi (1988) proposes that flexibility may be enabled by response thresholds to

stimuli that change with age (or size). Hence foragers are those individuals with a lower threshold of response to foraging stimuli, such as solicitation of trophallaxis from other workers. Thus the pattern of temporal polyethism observed might result from age related modifications of thresholds for brood work (increasing threshold with age) and foraging (decreasing) amongst others. Hence Calabi's (1988) mechanism involves age related thresholds of response, whereas Oster and Wilson's (1978:144) mechanism involves age related probabilities of response to a given stimulus. Oster and Wilson consider flexibility on an evolutionary time scale (adaptive demography and selection acting on CDF); but their mechanism also allows some flexibility in the short term, in a similar way to Calabi's. Both models presuppose the existence of age related developmental mechanisms with respect to behaviour, and that workers will be aware of stimuli relevent to other task groups.

Tofts (1991) utilizes process algebra (Milner, 1990) to demonstrate that the correlation between age and task group in monomorphic ant colonies may be a purely emergent phenomenon. Tofts' ants are arranged as members of a production line in which they receive 'jobs' from one direction ('left'), perform (role specific) actions upon them, and pass the products on to the next elements of the line ('right'). His ants therefore interact in a linear series, as argued by Oster and Wilson (1978:10). Ants do the tasks appropriate to their position in the line; if an individual perceives a shortfall in incoming tasks or a surplus at their own level (i.e., they are unable to pass completed tasks on to the right), they move (thus switching tasks) in the appropriate direction. The speed of response of the system depends upon the degree of shortfall or surplus that stimulates a task switching response. Since ants are 'born' into the left end of the production line (they eclose in the brood pile), they take up tasks at that level (i.e., brood care). Tofts has shown that on average older ants will be found further to the right

of the production line (i.e., at the foraging end), since they are likely to have responded to more surplus at their own level and moved right more frequently than they have moved left, owing to the input of new work force at the left end only. Hence there will be a correlation between age and caste, without age being causally linked to task in any way.

Although on average, older ants will be found foraging and younger ants will be found further within the nest, there will be individuals, even in a colony that has not undergone particular loss of part of a caste, that perform tasks 'atypical' of their age (Tofts, 1991). There is considerable experimental evidence to support this (reviewed in Calabi 1988; Gordon, 1989; see also Sendova-Franks and Franks, 1992; Tofts and Franks, 1992).

Tofts' model assumes that the logical structure of interfaces between task groups is physically represented in ant nests; that is, that the physical neighbours of a particular task group are its neighbours in the production line. This appears to be the case for species with small colony size and structurally simple nests such as *L. unifasciatus*: Sendova-Franks and Franks (1992) report appropriate spatial arrangements and interactions between castes in this species.

2.2 Information Exchange in *L. acervorum*

2.2.1 Introduction

The points raised in the first half of this chapter, concerning the variety of forms of communication between ants and the nature of information exchanged, lead to the conclusion that the forms of communication between ants will be related

to some extent to colony behavioural structure, that is, the form of division of labour therein. If one wishes to investigate the possibility that high frequency bouts of activity may facilitate exchange of information between individuals, it is neccessary to consider what information individuals of *L. acervorum* colonies may need to exchange. Hence, we must consider the colony behavioural structure of *L. acervorum.*

We could imagine a strict equality of tasks among workers, where each individual is responsible for feeding and cleaning a few larvae and for maintaining its own nutritional status. In that case each worker would be involved in the same set of tasks; from grooming and feeding the brood to foraging for herself and that set of brood. We would expect such a colony to exhibit no division of labour; individuals work in noninteracting parallel-series, to use the terminology of Oster and Wilson (1978:12). Under these circumstances, workers would require no information concerning basic needs of the brood from other workers since they have direct experience of the food levels and hygiene requirements of their particular charges (although some information may still have to be exchanged, for instance concerning colony defence, and allocation of brood items to workers). In such a hypothetical serial colony, other forms of communication as introduced in Section 2.1.1 are not neccessary for brood raising: immediate signals mediating direct interactions between adults are not required, nor are modulators of long term behavioural change, as the colony functions with 'identical' adults. Such extreme serialization of labour might appear unlikely, but evolutionary arguments for the origin of eusociality via communual nest sharing from solitary ancestors implicitly assume such a behavioural structure in incipient colonies (for example, Evans, 1958, 1977). The existence of short term activity cycles in a hypothetical serial colony to facilitate information exchange would seem unlikely, and the case for activity bouts facilitating information exchange would be weakened for

colonies known to operate with serial labour.

However, if a division of labour exists whereby some workers are only involved in foraging whilst others only tend brood, information must be exchanged between task groups; foragers would require information on the food level of the colony, this could be obtained by sampling the nutritional status of sufficient brood care workers. Information on such colony conditions clearly requires frequent updating, and also direct interaction between individuals. It is therefore conceivable that activity cycles of the order of 20 minutes may be involved in the exchange of such information, if we accept the arguments presented (see Section 8.3.2) that synchronized activity may enable individuals to obtain more accurate information through increased sampling of their environment (other individuals) (Franks and Bryant, 1987).

Although other species of *Leptothorax* have been shown to exhibit a division of labour, there appears to be no direct study of *L. acervorum*. Herbers and Cunningham (1983) report a division of labour into three behavioural castes based on cooccurrence of behaviours in 30 minute samples in *L. longispinosus*. Behavioural acts were characterized into four roles in this species and in *L. ambiguus* (Herbers and Cunningham, 1983; Herbers, 1983) on the basis of frequencies of transitions between behaviours (see Section 2.2.3). In *L. longispinosus*, caste membership appears to be linked to size, foragers being significantly larger than brood workers, which were larger than the caste identified as social interactors. No such relationship between caste and size was reported for *L. ambiguus*, and division of labour in this species was less clear from act cooccurrence data (Herbers, 1983). Wilson and Fagen (1974) and Cole (1986) study aspects of behavioural repertoire and time budget in *L. curvispinosus* and *L. allardycei* respectively, but do not address the issue of labour division *per se*.

With the exception of *Amblyopone pallipes* (Traniello, 1978), division of labour appears to occur in all species studied to date (Hölldobler and Wilson, 1990: 320). In the light of these findings, it would be rather surprising if some degree of labour division was not found in *L. acervorum*. However, since a reasonable understanding of the behavioural structure of colonies may be fundamental to an explanation of activity cycles I undertook a minimal study of division of labour in *L. acervorum* colonies.

In order to demonstrate the need for regular short term information exchange, it is sufficient to demonstrate that all ants are not behaviourally identical; that is to say, at least two behavioural groups exist. Hence, the behavioural study described in the following section was designed with the aim of ascertaining whether a division of labour existed at all in *L. acervorum*, rather than quantifying exactly the number of castes, or how caste membership is determined.

2.2.2 Materials and Methods

Collection

Colonies of *L. acervorum* were collected from a site in the New Forest, Hampshire, England. The site is located close to Beaulieu Road Station, (OS Grid Reference: SU 349062). Colonies were located under small groups of *Pinus sylvestris* on raised sandy banks running parallel to the rail track (about 10 m away). This particular area of the New Forest is a patchy habitat of pine and deciduous trees (mainly beech and oak) scattered between areas of acid heathland (New Forest Heath). The habitat in which *L. acervorum* was found consists of raised hillocks of sandy soil each supporting 2 to 8 mature *Pinus* specimens, and running ap-

proximately 200 m in total length. This area appears to be separated from other apparently suitable habitat by at least 300 m , but no colonies of *L. acervorum* were found in these neighbouring areas. The raised banks supporting *L. acervorum* are well drained, although an extensive marsh runs perpendicular to the collection site, about 600 m away.

Colonies were found within fallen branches of *Pinus sylvestris*. Branch length ranged from approximately 20 cm to over 1 m. Although *L. acervorum* has also been reported nesting between loose bark on trees (Bolton and Collingwood, 1975:19; Collingwood, 1979:72), none were found so doing at this site. Colonies were most frequently found within wood that had fallen for some time, and showed evidence of activity by wood boring fauna. Colonies were not found in recently fallen or particularly hard wood, nor in sticks that were damp or rotten. These observations suggest that *L. acervorum* is utilizing burrows abandoned by wood boring insects to gain access to suitable nest sites, rather than creating them *de novo* by boring activity.

Colonies were located by flaking apart suitable sticks using a screwdriver or penknife, and collected using a pooter and paintbrush. Care was taken to collect all the brood, and the queen (although some colonies appeared to be queenless). Large colonies ramified along interconnecting tunnels for some distance (10 - 15 cm), so in order to find and obtain complete colonies it was neccessary to destroy quite a number of potential nest sites in a given locality. For this reason, I have avoided collecting extensively from the area, and have conducted many of the laboratory studies on colonies that were not fresh from the field.

Maintenance in the laboratory

The ants were allowed to emigrate from the collection bottles into artificial nests, made by sandwiching cardboard (0.2 cm thick) between glass slides (7.6 × 5.1 × 0.2 cm). The card was cut to allow a short (*circa* 0.6 cm), narrow (0.5 cm) passage into an interior chamber, maximum dimensions 6 × 3.5 cm; sometimes this nest space was smaller to allow the nest to take up the whole visual field when filming with certain lenses (see Chapter 3). The nest design was similar to that used previously at Bath University to house *L. acervorum* and *L. unifasciatus* and had proved successful for maintaining colonies for several years (N.R. Franks, pers. comm.).

Each nest was kept in a square petri dish 10 × 10 × 2 cm . The internal vertical sides of the dish were coated with Fluon (Northeast Chemicals, Woonsocket, R.I., USA), to discourage escape.

Water and forage were continually available in the petri dish; tap water in a clear plastic tube (5 × 1.5 cm) plugged with cotton wool, and food in the form of Bhaktar Whitcomb diet (carbohydrates and vitamins in an agar gel; Bhaktar and Whitcomb, 1970). Protein was provided in the form of live *Drosophila* larvae. Both food forms were replaced once per week; the water was inspected daily and replaced when neccessary.

Initially, colonies were maintained in a constant temperature unit at 10° C until April, when the temperature was raised to 25° C, with a light regime of 10L:14D (light commencing at 8.30 am GMT). Due to increased malfunction of the unit, colonies were moved out into the laboratory in July 1989. This resulted in variable summer temperatures (22-28° C) and laboratory lighting (approxi-

mately 9L:15D); light commencing *circa* 9am. There is evidence to suggest that these more variable conditions result in higher brood survivorship to eclosion, as well as (arguably) more closely matching natural conditions. An overwintering period, essential for correct development of sexuals (Buschinger, 1973) was provided; colonies were placed in a garden shed for at least 3 weeks commencing in the third week of December each year. Temperature in the shed was a slightly buffered version of external air temperature, rarely rising above 10° C, or below -1° C.

Census of colonies

Colonies were censused at quarterly intervals and after completion of experiments. The numbers of queens, workers and brood were counted; brood were divided into eggs, larvae and pupae. Larvae were divided into 3 size classes:

small.....< 1.5 mm; generally 0.5-1.3 mm

medium.....1.5-3 mm; generally 2.3-2.8 mm

large.....> 3 mm; generally 4-5.5 mm

Adults and brood of the smaller colonies could be counted directly in the nest, by eye or under a low power dissection microscope. This was not possible for larger colonies (> 100 workers) since worker number could easily be confused and larvae may be piled in several layers or obscured by adults. Such nests were emptied into a series of petri dishes by gentle tapping to facilitate counting of smaller numbers. I conducted most experiments on laboratory colonies within 6 months of collection from the field.

Experiment

From October to December 1989, I constructed time budgets and ethograms to ascertain the degree of task specialization in three colonies of *L. acervorum*. The colonies were allowed to emigrate into nests designed for easy extraction of individuals by an adapted pooter; a hole (1.5 cm diameter) was cut centrally in the upper glass slide of a standard nest and a third glass slide was placed on top to cover the hole. Ants were removed by revealing the hole and inserting a sufficiently narrow nozzled (*circa* 3 mm diameter) pooter into any part of the nest.

Individuals were placed in a petri dish over ice to slow their movement, and were marked with different colours of Tippex applied to their thorax with a fine entomological pin. Marked individuals were returned to the nest after one hour, allowing the mark to dry before other individuals could groom it off. The nest was left for at least 24 hours before observations commenced to allow recovery from disturbance. I observed marked focal ants for a period of 30 minutes both within and outside the nest using a dissection microscope.

Ten marked ants from three colonies (i.e., a total of 30 ants) were monitored: I recorded the start time of each behavioural act performed during the 30 minute period.

All observations were carried out between 11am and 3pm to reduce the likelihood of diurnal behavioural variation. From the 30 behavioural sequences obtained, ethograms (tables of relative frequency of occurence of each act) and time budgets (relative duration of each act) were constructed for groups of ants.

I assigned each ant to one of two groups, using the nomeclature of Otto (1958; in Sudd, 1982), in which colonies of *Formica sp.* appear to be structured into two basic castes, specializing on tasks within the nests (Innendienst) or externally directed, for example foraging (Aussendienst):

> INNENDIENST: ant remains inside the nest for complete observation period;
>
> AUSSENDIENST: ant spends all or some portion of observation period outside the nest.

Hence allocation to behavioural group was determined solely be whether the individual did or did not spend time outside the nest. I used this assignment procedure to avoid forcing the data into predetermined categories I wished to study (such as categorization using some measure of age; Sudd, 1982). Since some acts are by definition only performed outside the nes (Table 2.2.1), some aspects of my analysis do not enable rejection of the null hypothesis of no caste structure (see page 55).

2.2.3 Results

In Tables 2.2.1, 2.2.2, 2.2.3, t ime budgets for behavioural acts (as outlined in Table 2.2.1 and Table 2.6) are presented pooled for all ants, and for Innen and Aussen grouped ants. Figure 2.1 depicts the frequency distribution of total time spent on a selection of the more common behaviours.

Table 2.2.1

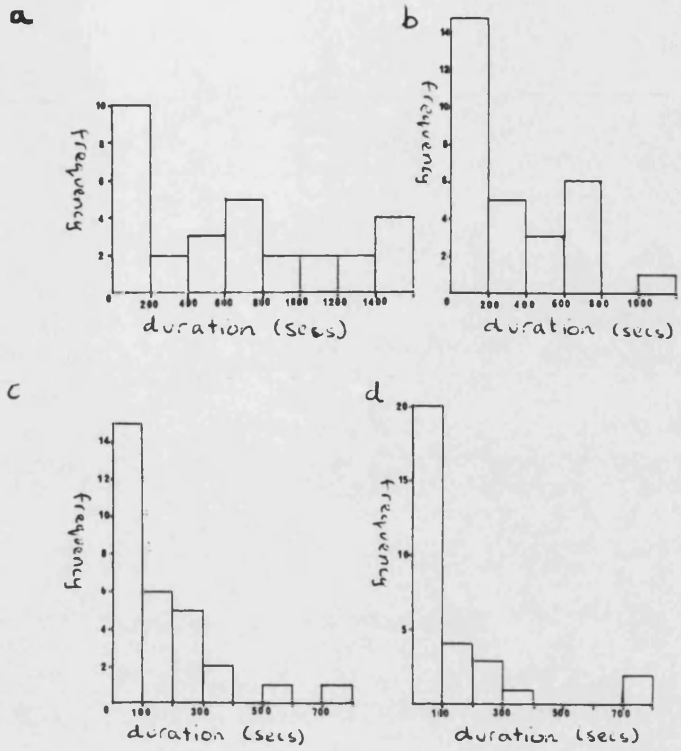

Figure 2.1: Frequency distribution of total time spent on various acts during 30 minute periods for 30 ants. a, Rest; b, Interim Movement; c, Groom Self; d, Brood Care.

Behavioural Act	*Abr.*	*n*	*Mean*	*S.d.*	*% total*
Rest	*Re*	*89*	*607.13*	*116.09*	*33.73*
Interim Movement	*IM*	*251*	*332.4*	*83.08*	*18.47*
Groom Self	*GS*	*142*	*156.2*	*72.98*	*8.68*
Brood Care	*BC*	*77*	*122.3*	*107.13*	*6.80*
Groomed by Worker	*GBW*	*16*	*40.2*	*127.75*	*2.24*
Groom Worker	*GW*	*17*	*15.47*	*50.33*	*0.86*
Exchange Food	*ExF*	*22*	*54.13*	*78.79*	*3.01*
Init. Ant. Cont	*IACW*	*65*	*17.77*	*31.79*	*0.99*
Receive Ant. Cont.	*RACW*	*54*	*18.77*	*27.58*	*1.04*
Carry nest Material	*CNM*	*10*	*5.13*	*46.84*	*0.29*
Collect Food/Water	*FdW*	*37*	*87.87*	*167.82*	*4.88*
Move Outside	*MO*	*100*	*246.43*	*142.34*	*13.69*
Rest Outside	*RO*	*13*	*67.73*	*115.08*	*3.76*

Time budget of Behavioural acts in three colonies of L. acervorum. A total of 30 individuals were studied for 30 minutes each. Abr: abbreviation of behavioural act; n: number of events; Mean, S.d.: mean duration of event (in seconds) and standard deviation; % total: % of total time budget occupied by act (for all 30

ants).

Table 2.2.2

Act	*n*	*Mean*	*S.d.*	*% total*
RE	*64*	*1000.38*	*373.29*	*55.58*
IM	*129*	*359.81*	*309.38*	*19.99*
GS	*58*	*161.81*	*217.53*	*8.99*
BC	*65*	*208*	*264.84*	*11.56*
GBW	*6*	*13.69*	*25.39*	*0.76*
GW	*6*	*15.31*	*39.71*	*0.86*
ExF	*3*	*8.5*	*22.38*	*0.47*
IACW	*14*	*5.75*	*11.32*	*0.32*
RACW	*15*	*14.06*	*22.49*	*0.78*
CNM	*4*	*5.56*	*22.25*	*0.31*

Time budget of Behavioural acts of Innendienst 'task group' (n=16). Act: abbreviation of behavioural act; n: number of events; Mean, S.d.: mean duration of event (in seconds) and standard deviation; % total: % of total time budget occupied by act (for the 16 ants).

Table 2.2.3

Act	*n*	*Mean*	*S.d.*	*% total*
RE	*25*	*157.7*	*242.67*	*8.76*
IM	*122*	*301.07*	*252.02*	*16.73*
GS	*84*	*149.07*	*95.21*	*8.28*
BC	*12*	*24.42*	*82.63*	*1.36*
GBW	*10*	*70.57*	*215.98*	*3.92*
GW	*11*	*15.64*	*31.62*	*0.87*
ExF	*19*	*106.29*	*138.94*	*5.91*
IACW	*51*	*31.5*	*27.71*	*1.75*
RACW	*39*	*24.14*	*19.91*	*1.34*
CNM	*6*	*4.64*	*11.21*	*0.26*
FdW	*37*	*188.28*	*239.41*	*10.46*
MO	*100*	*528.07*	*471.55*	*29.34*
RO	*13*	*145.14*	*236.40*	*8.06*

Time budget of Behavioural acts of Aussendienst 'task group' (n=14). Act: abbreviation of behavioural act; n: number of events; Mean, S.d.: mean duration of event (in seconds) and standard deviation; % total: % of total time budget occupied by act (for the 14 ants).

To determine whether Innen and Aussen ants behave differently, I employed the G test for heterogeneity in the frequency of acts performed (Sokal and Rohlf, 1981: 722; as used for behavioural data by Fagen and Young, 1978). The contingency table and G statistics calculated are shown in Table 2.2.4, Table 2.2.5, Table 2.2.6. The results indicate that Innen and Aussen ants differ significantly in their frequency of performance of various behavioural acts ($G_{H,8df} = 111.46$; $P < 0.05$). Pooled over all acts, Aussen ants also perform significantly more acts than Innen ants ($G_{P,1df} = 5.27$; $P < 0.05$, Table 2.2.5). This is likely to be due to Aussen ants spending less time inactive (Tables 2.2.2, 2.2.3) and hence performing more acts each of short duration than Innen ants (individual resting acts are of relatively long duration : Section 5).

The total G statistic ($G_T = G_P + G_H$) indicates that there is overall heterogeneity in the table ($G_{T,9df} = 116.68$; $P < 0.05$); this heterogeneity is the result of both differences in the total number of acts performed, and differences in the frequency at which certain acts are performed. Table 2.2.6 indicates which behaviours contribute to this heterogeneity: significant differences are found between Innen and Aussen groups performing (Re+RO), GS, BC, ExF, IACW and RACW(A fold out table at the end of this chapter lists behavioural acts and abbreviations referred to in this chapter) ($P < 0.05$ for these individual G_cs for behavioural categories). In summary, the G test indicates that Aussen ants perform significantly less resting and brood care acts, and significantly more self grooming, antennal contact and food exchange acts. By definition, they also perform all acts that

occur outside the nest, including foraging, MO and RO. Other acts listed in Table 2.2.1 were not included in the analysis owing to their rarity of occurrence.

It should be noted that the G tests alone cannot be taken as evidence for a division of labour, in that the frequency of performance of all acts cannot be assumed to be independent of the categorization with respect to exiting or not exiting the nest.

TABLE 2.2.4

Act	*n=16 Innendienst*	*n=14 Aussendienst*	*Row Totals (=T_r)*
RE	*64*	*25*	*89*
IM	*129*	*122*	*251*
GS	*58*	*84*	*142*
BC	*65*	*12*	*77*
GBW	*6*	*10*	*16*
GW	*6*	*11*	*17*
ExF	*3*	*19*	*22*
IACW	*14*	*51*	*65*
RACW	*15*	*39*	*54*
Column Totals(=T_c)	*360*	*373*	*733 (=N)*

Observed frequencies of behavioural acts for Innendienst and Aussendienst task groups; raw data for G_H tests. Acts are abbreviated as in Table 2.2.1.

Table 2.2.5

Statistic	*Value*	*Df*
G_H	*111.46 (*)*	*8*
G_P	*5.27 (*)*	*1*
G_I	*see Table 2.2.6*	*1*
G_T	*116.68(*)*	*9*

Values of G statistics from heterogeniety tests on frequency of behavioural act occurence in two task groups (see Table 2.2.4). (*) indicates significant departure from null hypothesis ($P<0.05$) G_H:H_0, task groups do not differ in frequency of behaviours; $X^2_{0.05,8df}=15.5$. G_p:H_0, task groups perform the same number of acts; $X^2_{0.05,1df}=3.8$. For details of calculations see Table A.0.2

Table 2.2.6

Act	*(1df) G_I*
RE	*12.87 (*)*
IM	*0.38*
GS	*8.89 (*)*
BC	*33.37 (*)*
GBW	*1.62*
GW	*2.24*
EXF	*15.21 (*)*
IACW	*27.68 (*)*
RACW	*14.49 (*)*

G test for individual behaviours (null hypothesis: acts are performed with equal frequency by each Group. (*) indicates significant departure from null hypothesis for given behavioural acts.

The inferences concerning frequency of act performance are supported by the data for act duration (presented as % total time budget in Tables 2.2.2, 2.2.3). Innen and Aussen workers spend similar amounts of time on IM (19.99% and 16.73% respectively), but differ markedly in their proportion of rest (55.58% and 16.83% respectively). Both groups are occupied by GS for similar total durations (8.99% for Innen and 8.28% for Aussen), but the frequency of grooming acts (Table 2.2.4) suggests that Aussen ants perform more frequent, but shorter grooming acts. Aussen ants also appear to spend more time on social acts (IACW, RACW; 3.09% compared to 1.1%). As a consequence of differences in time spent inactive, Aussen ants appear to spend more time performing task specific acts: approximately 51% (BC, FdW, P, ExF) compared to 13% (BC, ExF) for Innen ants. Figure 2.2 a-f depict the total duration during the 30 minute observation periods of more frequent acts (log_{10} transformed for representation of acts of short duration) for a selection of individuals typical of their assigned category.

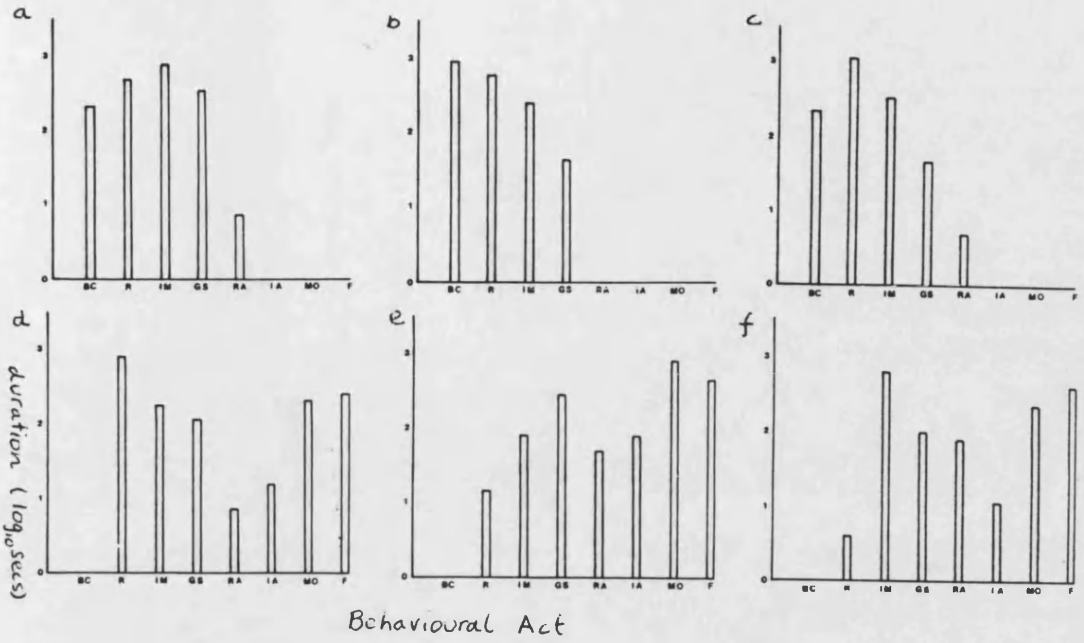

Figure 2.2: Graphical representation of time budgets from selected individuals for various behaviours. Horizontal axis: abbreviated behavioural acts; Vertical axis: log_{10} transformed duration of behaviour (maximum $log_{10}(1800) = 3.256$). Diagrams a-c: individuals classed as Innendienst; Diagrams d-e individuals classed as Aussendienst.

One difference between Innen and Aussen ants of interest from the perspective of information exchange between groups is their differing propensity to perform brood care. Figure 2.3 illustrates this phenomenon: only 2 out of 14 ants that spent time outside the nest also performed brood care. In Tables 2.2.7 and 2.2.6, the association between brood care and Innendienst is shown to be significant ($X^2_{0.05,1df} = 13.39$; $P < 0.05$). This result (as with others in this chapter) can only strictly be interpreted as indicating that individuals observed to leave the nest in a 30 minute period are unlikely to engage in brood care during that time. From Fig 2.3, such individuals have time to perform brood care (they are out of the nest for no more than 50% of the period), however these results cannot be taken to imply a division of labour that is stable in the long term. This aspect of the data interpretation will be explored in Section 2.2.4.

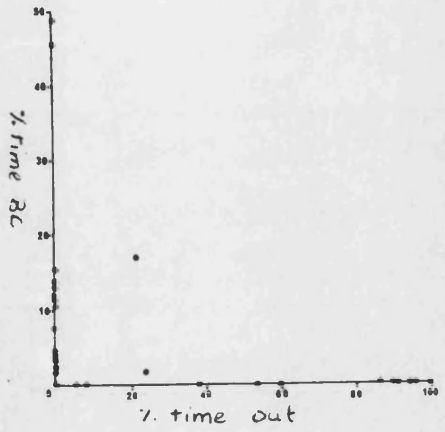

Figure 2.3: Negative association between brood care and Aussendienst: for each individual, % time spent outside nest (horizontal axis) is plotted against % time spent performing brood care. Only 2 individuals out of 30 perform in both catagories.

Table 2.2.7

	Innen	*Aussen*	*Row Totals*
BC	*13(8)*	*2(7)*	*15*
No BC	*3(8)*	*12(7)*	*15*
Column Totals	*16*	*14*	*30*
χ^2		*13.39 (1df)*	

Association between brood care and Innendienst. For each group, observed frequencies of ants that do or do not perform brood care are given. Figures in parentheses: expected frequencies based on Model 1 (Sokal and Rohlf, 1981: 773; no marginal values). χ^2 *test reveals a significant (P<0.05) association between task group and performance of brood care:* ($X^2_{0.05.1df} = 3.8$).

Transition frequencies between behavioural acts are shown in Tables 2.2.8, 2.2.9 2.2.10 for all ants, those grouped as Innen and those grouped as Aussen respectively. Association between behavioural acts, thus indicating one step transitions that occur more or less frequently than expected from the null hypothesis of no association, were determined by χ^2 analysis.

The null hypothesis of no association was adjusted to take account of transitions which by definition could not occur (see Section 2.2.4). This procedure, the expected frequencies and χ^2 values for individual cells are given in Table A.0.3.

Table 2.2.8

Act	*ReR0*	*IM*	*GS*	*BC*	*ExF*	*IACW*	*RACW*	*FdW*	*MO*	*Tot*
ReRO	*x*	*47+*	*9*	*6*	*0*	*5*	*10*	*2*	*5*	*84*
IM	*49+*	*x*	*63*	*33*	*1-*	*27*	*23*	*x*	*14-*	*210*
GS	*14*	*65+*	*x*	*9*	*0-*	*4-*	*4-*	*9*	*25*	*130*
BC	*11*	*26*	*11*	*24+*	*0*	*0-*	*2*	*x*	*x*	*74*
ExF	*1*	*13+*	*2*	*0*	*0*	*1*	*3*	*0*	*1*	*21*
IACW	*3*	*25*	*4-*	*1-*	*6+*	*1*	*1*	*4*	*20+*	*65*
RACW	*4*	*25+*	*2-*	*1*	*14+*	*0-*	*x*	*0*	*8*	*54*
FdW	*2*	*x*	*11*	*x*	*0*	*1*	*0-*	*x*	*18+*	*32*
MO	*5-*	*11-*	*27*	*x*	*0*	*25+*	*10*	*17+*	*x*	*95*
Totals	*89*	*212*	*129*	*74*	*21*	*64*	*53*	*32*	*91*	*765*

$X^2 = 438.19$ *(55df)*

Single step transition matrix for behaviours performed by all 30 ants. x implies no transition possible, individual transitions that occur more often than the null hypothesis of no association (P< 0.05, 1df) are marked as +; those that occur less frequently by are marked as -. For details of null hypothesis see Table A.0.3. χ^2*=438.19 (df=55) for summed cell values indicates significant heterogeneity.*

Table 2.2.9

Act	*Re*	*IM*	*GS*	*BC*	*IACW*	*RACW*	*Totals*
REe	*x*	*37+*	*5*	*6*	*0*	*6*	*54*
IM	*32*	*x*	*36*	*27*	*13+*	*8*	*116*
GS	*8*	*36+*	*x*	*8*	*0*	*1*	*53*
BC	*11*	*19*	*11*	*20+*	*0*	*2*	*63*
IACW	*0*	*10+*	*1*	*0*	*3+*	*0*	*11*
RACW	*3*	*11+*	*1*	*1*	*0*	*0*	*16*
Totals	*54*	*113*	*54*	*62*	*13*	*17*	*313*

$X^2 = 86.66$ *(22df)*

Single step transition matrix for behaviours performed by Innendienst ants. x implies no transition possible, individual transitions that occur more often than the null hypothesis of no association (P< 0.05, 1df) are marked as +; those that occur less frequently are marked as -. For details of null hypothesis see Table A.0.4. χ^2*=86.66 (df=22) indicates sinificant heterogeneity.*

Table 2.2.10

Act	*Re*	*IM*	*GS*	*IACW*	*RACW*	*FdW*	*MO*	*Totals*
Re	*x*	*10*	*4*	*5*	*4*	*2*	*5*	*30*
IM	*17*	*x*	*27*	*14*	*15*	*x*	*14-*	*87*
GS	*6*	*29+*	*x*	*4-*	*3*	*9*	*25*	*76*
IACW	*3*	*15*	*3*	*1-*	*1*	*4*	*20+*	*47*
RACW	*1*	*14+*	*1*	*0*	*0*	*0*	*8*	*24*
FdW	*2*	*x*	*11*	*1-*	*0-*	*x*	*18+*	*32*
MO	*5*	*11-*	*27*	*25+*	*10*	*17+*	*x*	*95*
Totals	*34*	*79*	*73*	*50*	*33*	*32*	*19*	*391*

$X^2 = 119.72$ *(30df)*

Single step transition matrix for behaviours performed by Aussendienst ants. x implies no transition possible, individual transitions that occur more often than the null hypothesis of no association(P< 0.05, 1df) are marked as +; those that occur less frequently are marked as -. For details of null hypothesis see Table A.0.5. χ^2*=119.72 (df=30) indicates significant heterogeneity.*

The summed χ^2 value for each matrix indicates overall departure from the null hypothesis ($P<0.05$, see Tables 2.2.8, 2.2.9, 2.2.10). This heterogeneity is the result of a number of transitions which are under- or over-represented compared to the null expectation. Figures 2.4a-c depict those transitions between acts that were significantly ($P<0.05$, 1df) and positively associated, for all observations, those pertaining to Innen , and Aussen respectively.

To some extent, positive associations occur between acts that other observers have suggested co-occur to constitute a role (Oster and Wilson 1978:122; Herbers and Cunningham 1983, Herbers 1983). For instance, BC acts are significantly associated only with themselves (Figure 2.4a,b), and foraging acts (FdW) precede and follow MO more often than would be expected from random occurrence ($P<0.05$; Figure 2.4a,c). Although suggestive of division of labour into roles (Herbers, 1983; Oster and Wilson 1978:122), these results should be taken only as weak evidence for the existence of castes (see Section 2.2.4), since they refer only to one step associations.

The overall structure of each graph however may yield insight into aspects of the short term behavioural organization of individuals and colony. Firstly, structural differences between Figure 2.4b and 2.4c (i.e., that different acts are associated) suggests that the Innen and Aussen groups of ants are behaving (in the short term) in different ways. One similarity between each group is the position of IM: it is rarely associated as the preceding partner of other behaviours, but is frequently associated as the following element in a transition. For example, in Figure 2.4b: $P<0.05$ for Re-IM, GS-IM, RACW-IM and IACW-IM. This suggests that IM tends to intervene between other behaviours (there are no one step associations between the partners of IM apart from RACW-ExF in Figure 2.4a).

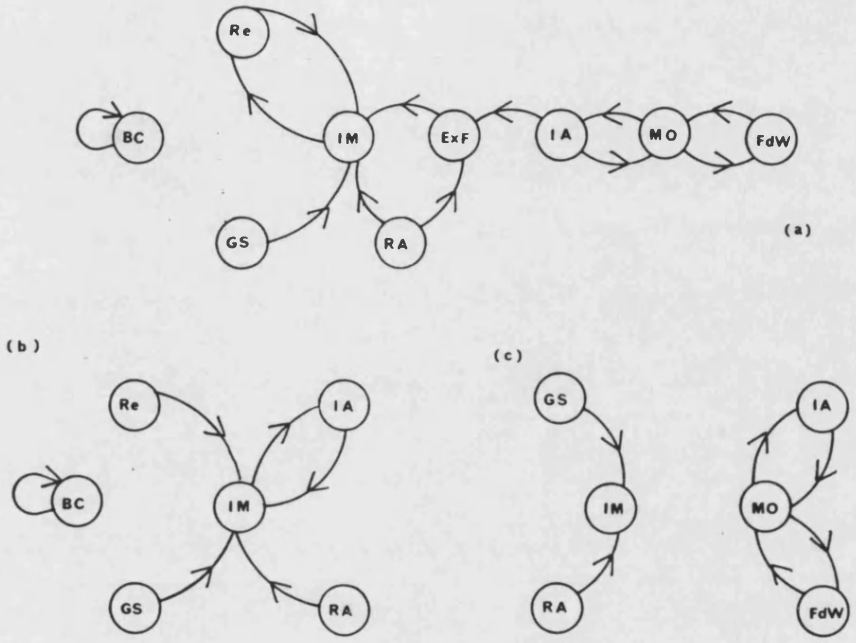

Figure 2.4: Graphical representation of positively associated single step transitions between behavioural acts. a: all ants (n=30); b, Innendienst (n=16); c, Aussendienst (n=14). Positive transitions are calculated on the basis of $X^2_{0.05,1df}$ from the frequencies given in Tables 2.2.8, 2.2.9 and 2.2.10. Acts are represented by a circled abbreviation. The direction of transition that is positively associated is indicated by the direction of the arrows between acts.

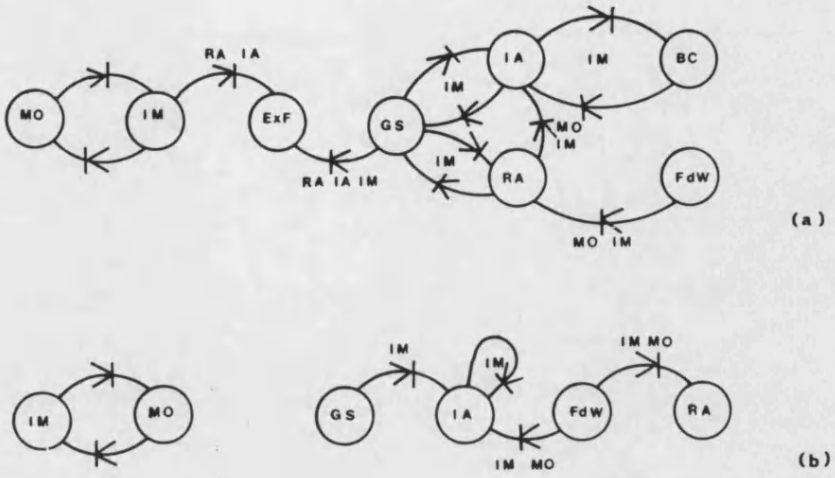

Figure 2.5: Graphical representation of negatively associated single step transitions. The direction of the transition that occurs less frequently than expected is indicated by a blocked arrow (a $\not\rightarrow$ b , b follows a less than expected). a, all ants; b, Aussendienst group. No significant negative transitions were found for the Innendienst group. Acts *not* circled indicate likely intervening acts, from inspection of Figures 2.4a-c.

Negative associations (Figures 2.5a,b) can be interpreted as resulting from one of three factors:

1. non cooccurrence resulting from behavioural differences between ants (individuals that perform A do not perform act B);
2. acts A and B are performed by the same individual, but act C intervenes between A and B;
3. definitional problems that were not taken account of by the null hypothesis.

Hypothesis (1) is not supported for any of the negative transitions; for each pair, both elements co-occur for individuals in the 30 minute sample (Table B.0.2). The transitions MO-IM and IM-MO might be interpreted under hypothesis (3); since MO can only follow IM directly (and *vice versa*) when the individual exits

(or enters) the nest. Negative association of this pair thus indicates that ants tend not to enter and exit the nest without performing a number of intervening behaviours outside. The remaining negative associations are likely to result from factor (2). On examination of Figures 2.4a-c, under-representation of these pairs can be explained as the consequence of intervening social interactions (IACW, RACW) or movement outside (MO) or in particular movement inside (IM) the nest (see also Figures 2.5a,b).

2.2.4 Discussion

Evidence for the division of labour

The data presented concerns a small number of ants (n=30) observed for a short time period (30 minutes). Although I provide no direct evidence for the existence of castes, in the sense that no data pertain to the long term behaviour of individuals, the data do support the notion of 'task groups' (Section 2.1.2), and do not contradict the suggestion that a longer term division of labour exists. The supposition of Otto (1958) that individuals occupy one of two roles Innendienst and Aussendienst is supported in that when ants are assigned to such groups on the basis of one rule (does or does not leave the nest), significant differences in the frequency of performance of various acts are revealed (G test; $P<0.05$; Tables 2.2.5, 2.2.6). In particular, brood care acts are performed by a subset of ants, which generally does not intersect with the subset of ants that leave the nest (χ^2 test; $P<0.05$; Tables 2.2.7 and Figure 2.3).

Time Budgets

The overall differences in time budgets between Innen and Aussen groups are in line with the statistically demonstrated differences in frequency of act performance. Aussen ants appear to spend more time performing social behaviours and task specific behaviours, and less time inactive. Time spent on IM and GS is similar in both groups.

Herbers (1983) reported that *L. ambiguus* individuals spend 68% time resting, and Herbers and Cunningham (1983) found that 67% time was spent resting in *L. longispinosus.* I found that overall *L. acervorum* spent 38% time inactive, although my sample size is half that of the other studies. This difference may be the result of my lower sample size, or slightly different classifications of behavioural act, but warrents further investigation into the parameters affecting activity (for example, temperature; brood/worker ratio). Personal observations (myself, A.B. Sendova Franks, N.R. Franks) have suggested that *L. acervorum* colonies are more easily disturbed, and individuals move faster than in colonies of myrafant species (*L. unifasciatus* and *L. tubero- interruptus*). One possible explanation for the relatively high level of activity in my colonies may be that focal ants were marked, which may cause increased agitation.

It is also possible that 'resting' is underestimated due to the difficulty in detecting the onset of this act. If a motionless individual starts to move, it has clearly changed state from inactive to active, and can be relatively quickly recorded as doing so. However, if an active ant stops moving, the observer must then decide at what point lack of movement indicates a change of state to inactivity, rather than a brief pause in movement (Reynolds et al., 1987).

The large proportion of time spent inactive is in itself of interest. This phenomenon has been suggested as indicating that under normal circumstances, there is a surplus work force in comparison to colony labour requirments (Wilson, 1971:341). This surplus might be neccessary to allow quick colony response to changing environmental conditions, or 'catastrophes' such as nest destruction or invasion by predators (Michener, 1964; Lindauer, 1961). It should also be pointed out that the high level of adult inactivity may be a behavioural artifact of laboratory based colonies, for which food is available *ad libitum* in close proximity to the nest and which are not exposed to predators or alien colonies. This possibility is likely to remain unresolved since it is not possible to conduct noninvasive studies of behaviour within field nests.

Ants that remain within the nest spend more time performing IM (20%) than they do 'working' (13%). That IM is so common requires explanation, since arguments involving concepts of efficiency (Charnov, 1976; Krebs and Kacelnik, 1991; Herbers, 1981a) would suggest that IM would be reduced if it was not necessary or beneficial (since it entails an energetic cost). IM may enable individuals to relocate to other tasks, or assess work loads within tasks they currently perform (see also Chapter 8), as well as facilitating direct communication between individuals.

Transitions between acts

One step transitions between behavioural acts have also been used as evidence of division of labour (Herbers 1983; Herbers and Cunningham, 1983). In Herbers (1983) (Herbers and Cunningham, 1983), acts were classed into a number of groups thought by the observer to constitute roles (or tasks), and the transitions between roles are analysed. Herbers (1983) found positive associations (χ^2 test)

between transition pairs of an element to itself, and negative associations between elements and non-self elements. This was taken as evidence for the existence of caste, since it implies that individuals performing acts within one role tend to remain performing acts in that role and switch roles less often than expected by chance. As Herbers (1983) points out, such statistical relationships may result from the intervention of other acts; the transitions between elements may not be direct but occur nonetheless. Hence one step transition probabilities measure aspects of behavioural organization on an even shorter time scale than that of 30 minute sampling.

By amalgamating acts into roles, Herbers avoids the problem of constructing a more complicated null hypothesis to take account of definitional problems. When individual acts are tabulated separately, some acts cannot be immediately repeated, since a different act must intervene for the observer to be able to detect the ending of the first act (Tables 2.2.8, 2.2.9 and 2.2.10: Re, IM, GS, FdW, P). Other acts such as grooming a brood item and IACW have natural discontinuities which permit their sequential occurence, for instance switching to a different brood item or antennating a different ant. Other pairs of acts cannot be connected directly for reasons involving their spatial location: BC-FdW cannot occur since BC occurs inside the nest, and FdW outside. The two acts must be minimally connected as follows: BC-IM-MO-FdW. The study of Herbers (1983) may also involve problems of this latter type: although elements consist of more than one act, elements constituting the role of 'brood care' rely on connection through 'personal behaviour' (containing 'movement inside nest') to reach 'provisioning' (movement outside nest and foraging behaviours). Indeed, the amalgamation of acts itself may decrease the ease of interpretation since the observer is required to decide which acts (already imperfectly defined objects) belong to which 'roles' (themselves determined by the interpretation of the observer).

When transitions are analysed between acts rather than groups of acts, it is possible to reason more clearly about the structure of sequences of behaviour, and identify key behaviours which may otherwise be obscured by the effects of grouping. Oster and Wilson (1978:122; also Hölldobler and Wilson 1990:300) suggested that transition probabilities between single acts combined with information on act duration might be used to define roles as groups of acts strongly connected together with weak connections between other such groups, thereby avoiding the neccessity for *a priori* definitions by the observer. A transition based definition of role has advantages of objectivity and repeatability over the somewhat subjectively defined 'task', however the data presented in this chapter are not of sufficient quantity or quality to warrent a formal analysis in this fashion.

The transition structure presented in Figure 2.4 do suggest that the definition of role may be difficult to implement. Apart from BC, all the acts analysed are linked by one or more significant positive transitions (Figure 2.4a), making it difficult to subdivide the set into roles. However, individual acts tend to be linked to few others, producing a relatively linear transition structure. The position of acts along this structure is consistent with the concept of tasks being organized as a production line (Tofts 1991; Tofts and Franks, 1992). That is, individual acts that clearly represent work or 'jobs' (e.g., BC, ExF, FdW) are not strongly connected to each other, but are connected in a linear manner via other acts that may be considered as mediators of interaction (IM, IACW, RACW). On this basis, the simplifying assumption of a linear arrangement of tasks with workers sampling information at the interface between tasks (Tofts, 1991a, 1991b) appears to be plausible.

Information exchange

The results presented suggest that division of labour occurs in laboratory colonies of *L. acervorum*, at least in time frames of the order of the sampling period (30 minutes). Individuals do not appear to perform all colony tasks, and are thus not arranged in parallel-series (Oster and Wilson, 1978:12). Hence there appears to be some neccessity at the colony level to exchange information between individuals, in order that work levels be gauged and demands from other castes responded to. Although castes may not be stable over hours or days it seems unlikely that individuals switch task so frequently that information on work levels is unnecessary. Hence it appears reasonable to investigate colony activity patterns in relation to information exchange, as the need for the latter is established.

The structure of one step transitions between acts suggests that information exchange may occur at the interfaces between task specific behaviour; the behaviours mediating this exchange being movement inside and outside the nest, and antennal contact. RACW and IACW are clearly associated with exchange of food inside the nest between workers. Frequent antennation also occurs outside the nest in association with MO, and may serve a defence function related to recognition. Inside the nest, IM appears to intervene between other behavioural acts. It may be considered as a behaviour that enables information exchange in that it allows relocation of individuals to other tasks that are confined in space (Sendova-Franks and Franks, 1992), and hence enables individuals of different task groups to interact directly. IM may also allow individuals to sample work loads within their task group, for example by allowing inspection of other brood within the brood pile (see Chapter 8). The 'casual body contacts' mentioned in Section 2.1.1 are also likely to result from IM; although this 'communication' might not entail direct exchange of information, it may be involved in the forma-

Figure 2.6: Fold out table of abbreviations of behavioural acts used in Chapter 2.

tion of temporal patterns of colony activity.

Behavioural Act	Abr	Notes
Rest	Re	Stationary, no head or antennal movement
Interim Movement	Im	Lcomotion within nest
Groom Self	GS	
Brood Care	BC	Feed, clean, inspect move or feed from any brood item
Groomed by Worker	GBW	
Groom Worker	GW	
Exchange Food	ExF	Receive or donate food by trophallaxis
Init. Ant. Cont.	IACW	Initiate antennal contact: approach other and antennate
Receive Ant. Cont.	RACW	Receive antennation from another
Carry Nest Material	CNM	
Collect Food/Water	FdW	Collect food solid or Bhaktar & Whitcomb or drink
Move Outside	MO	All locomotion outside the nest
Rest Outside	RO	Stationary outside the nest

Chapter 3

The Development of an Image Processing System

3.1 Introduction: The Principle

Pictures taken using a monochrome video camera consist of pixels: small squares of varying shades of grey. The picture can be converted to a binary image (black and white only) by the process of digitization using appropriate hardware and software. Thus monochrome pictures of ant colonies can be converted to a binary picture consisting of black ants against a white background (Figure 3.1). Such images are stored in the computer memory as a binary code ("1" for white; "0" for black; 32000 pixels in all for this system). By comparison of the binary code for two pictures, one can measure the amount of difference between two frames by scoring the number of mismatches of pixel values. In theory, the greater the number of ants that have changed position between two frames, the greater the score of unmatched pixels between those frames. In practice, the relationship between number of pixel changes and number of changes in ant positions will not be linear; in order to equate colony activity level with pixel change, we must

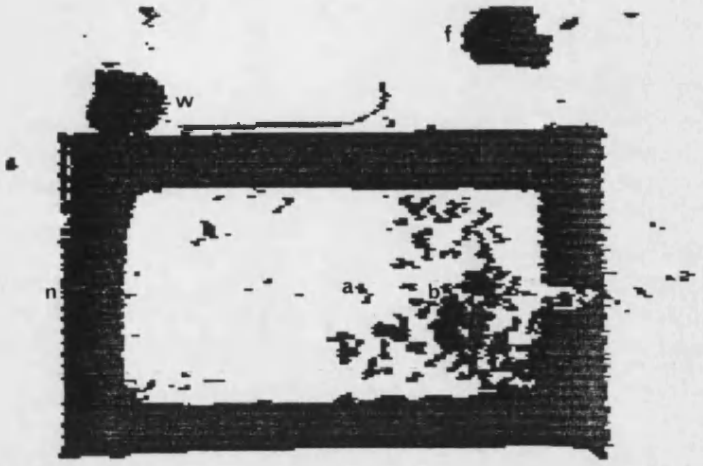

Figure 3.1: Digitized image of an ant nest: showing black ants (for example (a)) on a white background. The nest frame (n), food and water tubes (f,w), and dense brood pile (b) also appear black.

examine a number of logical steps.

Change in position equates to movement

Let us first consider the case of consecutive images taken of a single black object against a white background. If the object moves, pixel colour may change in one of two ways: white to black (pixels that previously represented the background now represent a portion of the object) and black to white (pixels that previously represented a portion of the object now represent background). If the object moves linearly and slowly with respect to the time interval between frames (such that the object is not completely displaced relative to its position in the previous frame), there will be a linear relationship between the amount of change in position, or the speed of movement, and the number of pixel differences (Figure 3.2 a, b). In effect, the number of pixel changes represents twice the area of positional change of the object; not only is change recorded at the leading edge of the object as it invades "new"space (white to black changes), but also where the receding

edge retreats from "old " space. If the object moves linearly and relatively fast (at or above the threshold speed V_T at which the area of overlap of black pixels between consecutive frames is zero; see equation 2.i below), the number of pixel changes remains at the maximum, representing twice the area of the object. Here, pixel change will not relate to "amount of movement", *vis a vis* speed (i.e., distance moved between consecutive frames): the area of positional change of a moving object can be no greater than the area of the object itself (see Figure 3.2 c, d). The situation is further complicated when considering rotational movement: if an object perfectly circular in outline were to rotate about its centre of gravity, no pixel changes would be recorded. For non circular objects, some pixel changes will be recorded, but positional change will be underestimated compared to the case for translational movement.

Eqn 2.i ..... Threshold speed V_T:

$$V_T = \frac{\text{max. length of object}}{\text{time interval between frames}}$$

Let us now consider images of more than one black object against a white background. If all objects move relatively slowly (below V_T), and do not come to occupy positions that were occupied by another object in the previous frame, the number of pixel changes will be positively and linearly related to amount of movement (in terms of speed of movement and number of moving objects), reaching a maximum when all objects move at or faster than the V_T. When objects come to occupy positions occupied by others in the previous frame, the amount of movement will be underestimated by pixel change (even below V_T) in proportion to the degree of overlap between objects. Hence for objects with essentially random movement patterns in space, the degree of underestimation of movement will be proportional to the ratio of total object area to background

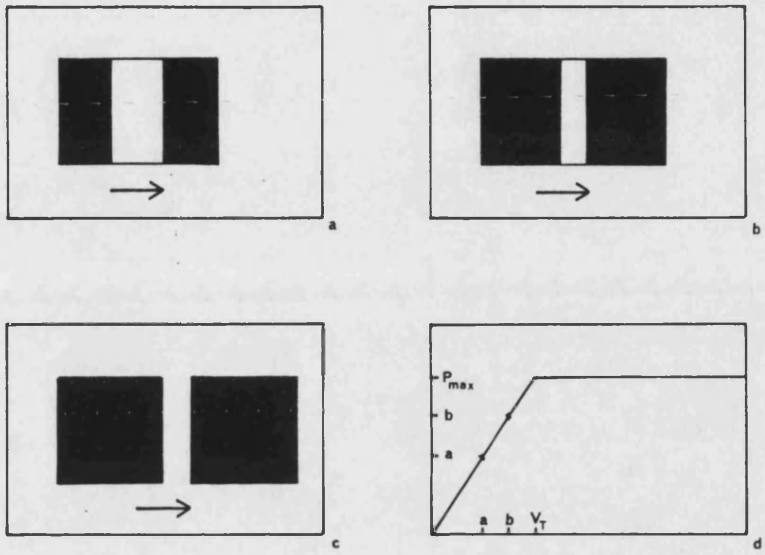

Figure 3.2: Relationship between the speed of movement of an object and the number of pixels that change state. (a) rectangular object moves half its length between frames taken at t=2 and t=1. Shaded area represents pixels that change state. (b) object moves faster (relative to interframe interval). (c) object is completely displaced at t=2 compared to its position at t=1, its speed is higher than V_T. (d) relationship between speed and no. of pixels that change state, P_{Max}= 2 $\times$ number of pixels the object covers, points a and b are the changes that would occur in diagrams a and b.

area.

Movement equates to activity

By definition, an "inactive" animal does not move, unless it is pushed, pulled or carried by extrinsic factors. However, it is not clear that all activity requires movement. Purely physiological or neuronal activity need not involve movement, but ethological activity can only be recognized as a consequence of some movement pattern visible by an observer. In any case, the activity cycles under study in this thesis were originally recognized on the basis of movement activity only, by recognition of temporal patterns of movement from time lapse video recordings of ant colonies (behavioural acts of individuals remaining undefined) by other authors (Franks and Bryant, 1987; Franks et al., 1990a). Although clearly one would wish to investigate the precise nature of such activity when examining possible mechanisms and functions of colony activity patterns, for the purposes of ascertaining the existence and quantifying any such patterns movement itself is a sufficient parameter for mearurement, in the sense that activity of some sort is required to produce a change in position. Hence, for brevity, throughout this thesis I shall refer to "activity level"; by this I will mean "amount of positional change of objects within the field of view", unless otherwise stated.

Pixel differences are a measure of colony activity.

I have argued that amount of movement or positional change equates to activity, in a broad ethological sense appropriate to this study. I have also clarified how number of pixel changes may reflect positional change of objects. In summary,

three factors will lead to an underestimation of movement, and hence of colony activity:

1. speed of movement at or above the threshold speed (V_t above);
2. movement involving elements of rotation, or reversal of direction;
3. the effect of crowding, leading to overlapping of different objects between consecutive frames.

If we wish to equate colony activity with speed of movement of individuals, factor (1) can be resolved by taking frames at sufficiently short intervals. Individual *L. acervorum* ants frequently traverse the whole nest length (60 mm) in less than 10 seconds, and are approximately 5 mm long. Therefore in order not to exceed threshold ant speed, frames would have to be taken at less than 0.5 second intervals. This is beyond the capability of my equipment, and in any case entails a trade off between resolution of data and overall length of the time series gathered. Similarly, it is not possible to control for nonlinearities caused by factor (2), although since ants are not perfectly circular in outline, activity of this nature will at least be recorded to some extent. The effects of factor (3) are particularly difficult to quantify; they will entail an underestimation of activity in large colonies if housed in a similar sized nest as small colonies, and an underestimation of activity in portions of the nest such as the brood pile where ants are naturally more crowded. Assuming that the ants are arranged in two dimensions (so that it is not possible for ants to move on top of each other), which is essentially the case (see Chapter 2), activity will be underestimated in temporally "crowded" sequences, as well as spatially crowded areas: if many ants are moving at the same time, there is more likelihood that an individual's current

position will overlap with another's previous position, than if few ants are moving (since stationary ants exclude others from the space they occupy).

In summary, if we equate colony activity level with amount of colony movement, itself a function of the number of moving individuals and their speed, image analysis techniques based on counting the number of pixel changes between binary images will lead to an underestimation of higher activity levels. This somewhat inevitable feature of the system I have chosen for measurement is of prime importance in the choice of methods of data analysis. As explained elsewhere (Section 4.3.5); it leads to an avoidance, or at most tentative interpretation of amplitude of unmatched pixel counts as a measure of activity, and a concentration on methods that involve identification of minimal activity levels, and frequency of changes in activity level at a time scale that is long relative to the interframe period.

3.2 Development of an Image Analysis System

The system that I eventually arrived at was based on an earlier system developed by Sankson (1988). The focal ant colony is placed in a light tight cabinet with constant light and temperature regime, and filmed by a video camera (Panasonic model WV-1850/B) attached to a Zeiss photomicroscope. The monochrome camera output is fed directly into a Realitizer Video Digitizer (Print-Technik, Germany), attached to an Atari 1040ST microcomputer via the ROM cartridge port. Software provided by Print Technik enables the image to be digitized (converted to binary coded strings mapping the entire monitor screen into 32000 black or white pixels). Initially, the camera output was also fed to a Panasonic NV-8050 time lapse recorder, allowing simultaneous recording of the monochrome picture onto TDK HS E-180 VHS video tapes. Software developed by Sankson (1988) al-

lowed frames at preset intervals (minimum interval 10 seconds, maximum number of frames 260) to be digitized and saved to an Atari model SH204 hard disc. A further program written by Sankson (1988) calculates the number of unmatched pixels between consecutive frames.

This system was revised considerably to reduce the noise to signal ratio, which early experiments revealed to be in the range of 1:3. The revision of hardware and software will be discussed in the following sections, in relation to experiments carried out to test its performance.

3.2.1 Hardware revision

1. The video camera was originally used in conjunction with a Zeiss photomicroscope; some time was taken to experiment with lenses of different focal length to allow a sufficiently large field of view; it was eventually replaced with a Fujinon TV zoom lens attached directly to the camera.

2. The method of lighting was modified to reduce noise in the monchrome signal for digitization: previous methods of lighting from above with a "cold" light source produced an uneven light intensity across the background that resulted in ants being indistinguishable from background in some areas of the nest on digitization. A slight alteration in the angle of lighting resulted in differing digitized images, thus reducing repeatability and negating the possibility of comparison of the general level of unmatched pixels between runs. A consistent level of lighting was eventually achieved by placing the colony on top of an X-ray viewing box.

3. A new method of temperature regulation was required for use with lighting from below (originally, temperature was controlled to within 0.01° C of 25°

C by a thermostatically controlled base: Franks and Byant 1987; Franks et al., 1990a). The initial solution entailed placing the nest in a glass crystallizing dish of water, cooled by a metal element through which tap water was run. Maintenance of a stable temperature required continuous monitoring and adjustment of the water flow at the tap, and also disturbed the colony. Temperature control was achieved by moving the whole experimental system to a constant temperature room, running at 25° C and 50% humidity. Small oscillations in temperature were further reduced by bathing the chamber in glycerol contained in a clear glass crystallizing dish. Figures 3.3 a to c depict the evolution of the hardware system.

3.3 Tests of system

3.3.1 Methods

A number of preliminary experiments were carried out in conjunction with the development of the system to ascertain the existence and frequency of the supposed rhythms of activity (see Chapter 1). These are summarized in Table 3.3.1; the procedure is outlined below.

1. The colony, housed in an appropriately sized nest so that the nest occupied the whole field of view, and contained within a petri dish as described in Chapter 2, was placed in the light tight unit. The lights were switched on and the temperature control mechanism set in operation. The colony was allowed to acclimatize to these conditions for at least 1 hour before filming.

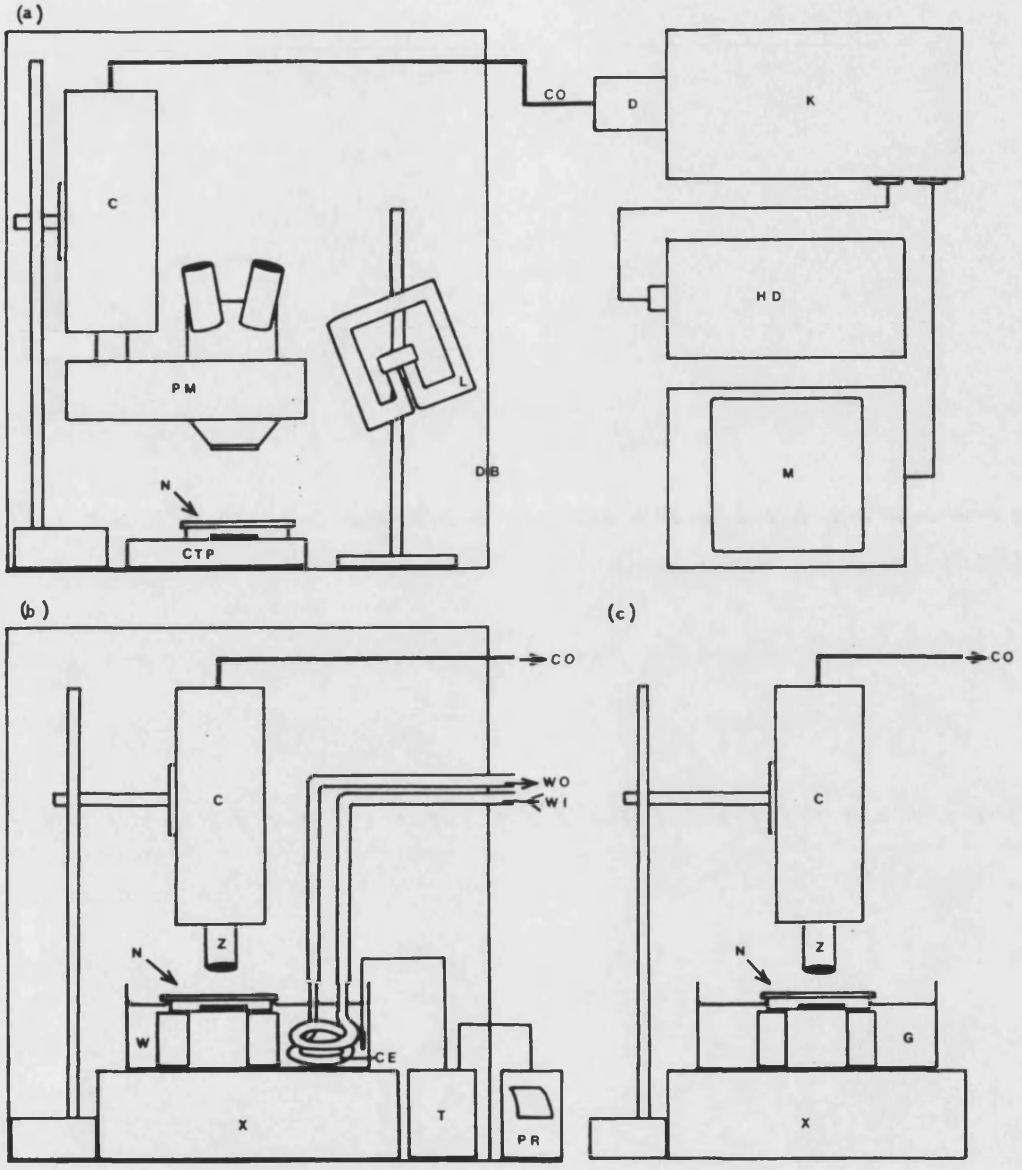

Figure 3.3: Development of the image analysis system and conditions: hardware. Initial system (a) through to final configuration (c) which was used for all further measurements (Chapter 4 onwards). System (c) was placed in a constant temperature room, 25° C, 50% humidity. Hardware is labelled as follows: C, camera; CTP, constant temperature plate; CE, cooling element; CO, camera output connection; D, digitizer; DB, dark box; G, glycerol; HD, hard disk; K, keyboard; L, light source; M, monitor; N, nest in petri dish; PM, photomicroscope; PR, pen recorder; T, digital thermometer; W, water; WO, water out; WI, water in; X, X-ray viewing box. Camera output was fed in all cases to the digitizer as shown in (a).

2. As filming began, the realitizer was set in operation using the GFABasic program AMMENDED.BAS (see Sankson, 1988), storing digitized images to the hard disc at preset intervals for a preset duration, simultaneous with any video recording. After initial trials, digitized frames were snatched at 1 minute intervals for 3 hours (180 frames).

3. After filming ceased, consecutive stored frames were recalled and compared by the GFABasic program COMP2.BAS. Owing to the slow speed of program execution in Basic, and inefficiencies in the encoding, comparison of all 32000 pixels between consecutive frames would take a prohibitively long time (*circa* 12 minutes). Hence the number of unmatched pixels was counted at a preset resolution or "step". At "step 10", one pixel in 10 is compared between frames; allowing a maximum of 3200 unmatched pixels per frame pair; this was deemed to be of sufficiently high resolution for my purposes.

Table 3.3.1

Run	*Rate*	*Time*	*Step*	*Colony or Pattern*	*Lens*	*Filming*	*System*
1	*6*	*1*	*10*	*pattern card*	*f100*	*no*	*a*
2	*2*	*1*	*10*	*dead ants(60)*	*f100*	*no*	*a*
3	*1*	*3*	*10*	*dead ants(60)*	*f300*	*no*	*a*
4	*1*	*3*	*10*	*dead ants(60)*	*zoom*	*no*	*a**
5	*1*	*3*	*10*	*Colony A (43)*	*f100*	*no*	*a*†
6	*2*	*3*	*10*	*Colony A (41)*	*f100*	*no*	*a*
7	*1*	*1*	*10*	*Colony A (43)*	*f100*	*no*	*a*
8	*1*	*1*	*10*	*Colony B (97)*	*f200*	*no*	*a*
9	*1*	*1*	*10*	*Colony6 (206)*	*f300*	*Yes x16*	*a*
10	*1*	*1*	*10*	*Colony6 (206)*	*f300*	*Yes x16*	*a*
11	*1*	*1*	*10*	*Colony E (137)*	*Zoom*	*Yes x16*	*a**
12	*1*	*1*	*10*	*Colony E* (96)*	*Zoom*	*Yes x16*	*b*
13	*1*	*1*	*10*	*Colony E (96)*	*Zoom*	*Yes x16*	*b*

Preliminary digitized runs. Summary of designs for preliminary experiments in Chapter 3. For each run, the number of frames captured per minute (Rate) and

total duration (Time) is shown. Step indicates the resolution of image analysis (see text for details). Colony: numbers in parenthesis refer to number of adults in the colony; E: starved for 18 days. Filming: x16 indicates filmed at 16 times natural speed (Time lapse). System: a-see Figure 3.3a; a*-as a but with zoom lense; a†-as a; natural light; b-see Figure 3.3b*

Noise measurement.

Noise, defined as the erroneous measurment of a Boolean parameter, can arise in two ways: the false conclusion of negatives (i.e., failure to record change in position, thereby underestimating activity), or false conclusion of positives (recording nonzero values of pixel changes when in reality no movement of focal objects has occured). False negatives, as discussed in Section 3.1, are likely to occur for a number of reasons and are extremely difficult to quantify. False positives can however be quantified by measuring the number of pixel changes that occur between images known to contain no moving features, as described below.

Noise levels were quantified by running the digitization procedure described above whilst filming white stationary cards or cards with black and white geometric designs. It was noted that noise levels were higher for designs with a greater length of interface between black and white areas. In order to quantify the relevant noise level when filming an ant colony, 60 dead ants were collected from midden piles and placed in a nest as used for live colonies. This "dead nest" control was filmed and frames digitized on three occasions.

Independent measures of activity

Initially, independent measures of activity were used in conjunction with digitization so that the two measures could be checked for correlation. On two digitized runs (LEPTO 4 & 5), the simultaneously recorded video film (timelapse, 48 times natural speed), was analysed as follows:

1. The number of ants entering and exiting the nest per minute was counted (3 hours duration as for digitization).

2. Ten ants selected at random were followed on the video screen for the 3 hour period, and their positions marked at minute intervals on an acetate sheet overlaying the screen. If the focal ant exited the nest during the observation period, it was subsequently ignored and the next ant entering the nest was followed (a procedure employed by Franks and Bryant, 1987; Franks et al., 1990a). For each ant, the distance between points at consecutive intervals was measured. Although this measure is liable to underestimate the total distance moved in 1 minute, it could be used to give some independent estimate of relative activity levels of individuals.

3. The 10 ants followed in 2. above were recorded at the end of each minute as being "active" (moving or stationary but involved in some activity such as grooming or antennating) or "inactive" (stationary and with no indications of performing behaviours i.e. "resting"; see Chapter 2).

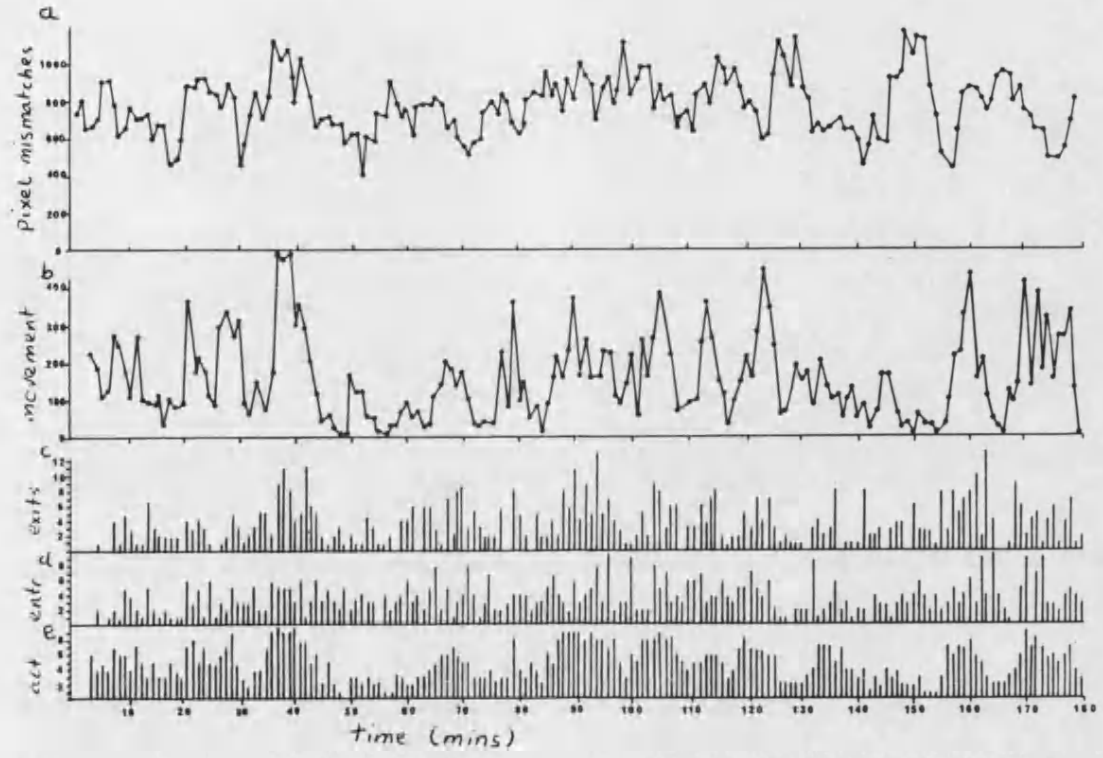

Figure 3.4: Time series of simultaneous measures of activity, LEPTO4. (a) automated measure (number of pixel mismatches between frames at one minute intervals) (b) summed movement of 10 focal individuals at one minute intervals (arbitrary units) (c) number of exiting ants per minute, (d) number of entrants per minute, (e) number active of 10 focal individuals.

3.3.2 Results.

Sample time series graphs of preliminary digitized runs are presented in Figure 3.4a and Figure 3.5a; I have chosen to present the results of runs 9 and 10 (LEPTO 4 & 5), where independent measurement of activity levels was also made (Figure 3.4b, Figure 3.5b). I have also presented a selection of time series from stationary image controls (Figure 3.6), for comparison.

Originally, standard techniques for time series analysis (such as autocorrelation and Fourier analysis; see Chatfield, 1984; Kendall, 1976) were to be performed on these preliminary data. However, for reasons discussed in Sections 3.4.2 and 4.5.2, this seemed inappropriate. It is still possible to test for randomicity of the

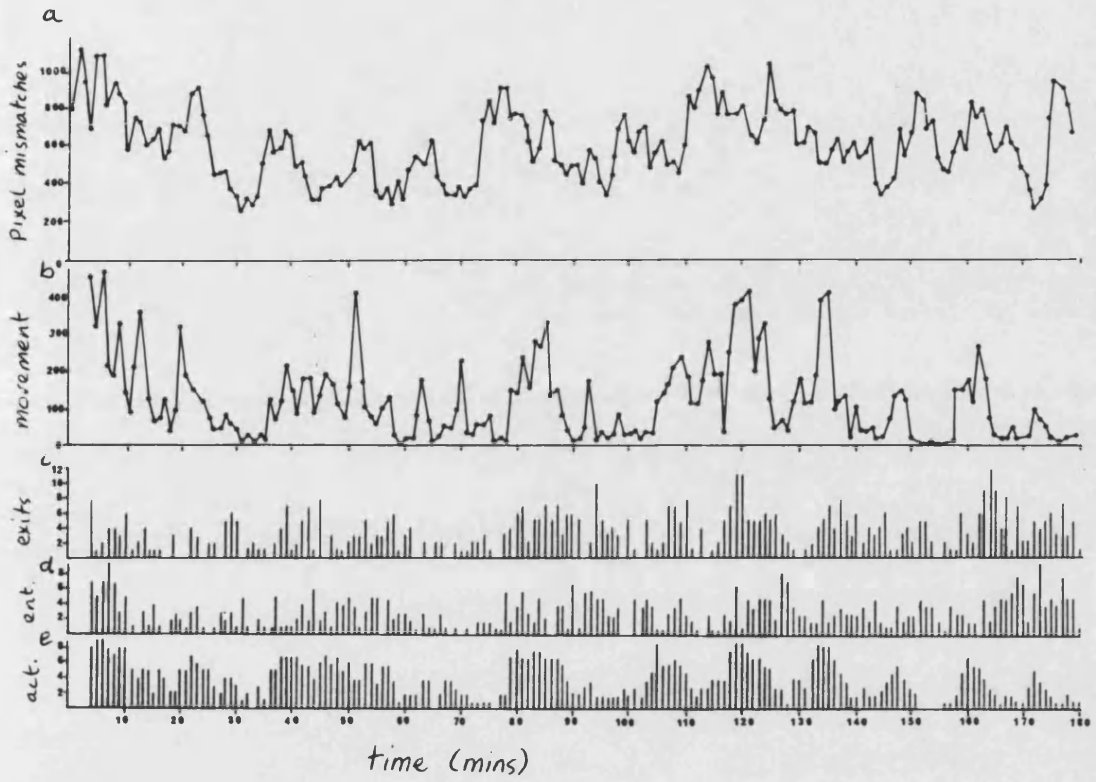

Figure 3.5: Time series of simultaneous measures of activity, LEPTO5. (a) automated measure (number of pixel mismatches between frames at one minute intervals) (b) summed movement of 10 focal individuals at one minute intervals (arbitrary units) (c) number of exiting ants per minute, (d) number of entrants per minute, (e) number active of 10 focal individuals.

time series data, by the method of Kendall (1976:22); the results are presented in Table 3.3.2; for methods see Table A.0.6.

Table 3.3.2 indicates that changes in counts of unmatched pixels are unlikely to have occured due to random effects ($P<0.05$) for all live colonies, but are likely to be random in all dead nest controls and stationary geometric designs. Similarly, the independent measures of colony activity, number of active ants (I9:ACT, I10:ACT), and total movement of focal ants (I9:SUM, I10:SUM), do not indicate random activity patterns, when analysed with respect to turning points.

3.3.3 Discussion

Visual inspection of the time series in Figures 3.4 ,3.5 (simultaneous measures of activity for LEPTO 4 & 5), appears to support the results of the turning point test, indicating nonrandom and somewhat pulsatile colony activity level. All data forms (apart from number of exits and entrants per minute), appear to consist of bouts of high levels of activity interspersed with periods of low level activity; activity levels do not appear to remain stable. The frequency of these bouts appears to vary considerably, but lies between 10 and 30 minutes; there is no apparent long term trend in levels across the complete time series, so these bouts do not appear to be connected with any long term rhythm in activity, such as *circa*dian rhythms. Broadly speaking, there appears to be reasonable correspondence in the times of high activity between the activity measures SUM and ACT. There is less of a correspondence between the number entrants per minute and any of the other measures of activity, suggesting that activity bouts are unlikely to be triggered by ants entering the nest. Notably, the time series for numbers of exits per minute (I9:EX; I10:EX) and entrance per minute (I9:ENT;

I10:ENT, Table 3.3.2) do appear to be random (falling within 95% confidence intervals for random expectation). Although turning point analysis indicates that the time series for exits varies randomly, strong peaks in exits do appear to correspond to colony activity peaks, possibly suggesting that exits may be triggered by increased activity.

When one compares the time series of the digitized data versus any manual observation measure of activity, there appears to be some lag in time between the recording of activity peaks by manual methods, and their occurence in the digitized data. It became apparent on inspection of the frame grabbing program that there were a number of software errors in the timing routine that rendered this program unusable. No account had been taken in the timing loop of the program for the time taken to grab a frame or store it to disc. The frame grabbing procedure required about 4 seconds, which was being added on to the preset time interval, hence a 3 hour data set in fact required about 3 hour and 12 minutes. Furthermore, as the hard disc began to fill throughout the data grabbing excercise, the time taken to write to disc increased, so that frames grabbed later in the sequence were taken at even longer intervals. These problems were alleviated and the efficiency improved markedly on a complete rewrite of this software. Figure 3.6a indicates a high level of noise; to whit, "dead nest"controls appear to be one third as "active" as live nests (maximum number pixel changes in live nests *circa* 1000, mean number pixel changes in controls *circa* 300). Such high levels of noise could easily swamp the signal when colonies are in a low state of activity. These difficulties were alleviated (Figure 3.6b) by writing new software for comparison of stored frames, as explained below.

Table 3.3.2

Run	*N*	*Turning Points*	*LCI*	*UCI*
1	*210*	*144*	*132*	*145*
2	*119*	*74*	*73*	*83*
3	*165*	*111*	*103*	*115*
4	*176*	*114*	*110*	*122*
5	*170*	*88**	*106*	*118*
6	*90*	*52**	*54*	*63*
7	*170*	*78**	*106*	*118*
8	*179*	*90**	*112*	*124*
9	*179*	*98**	*112*	*124*
10	*178*	*94**	*111*	*123*
11	*177*	*98**	*112*	*124*
12	*173*	*99**	*108*	*120*
13	*178*	*104**	*111*	*123*
I9:SUM	*170*	*100**	*106*	*118*
I9:EX	*145*	*99*	*90*	*101*
I9:ENT	*155*	*103*	*96*	*108*
I9:ACT	*134*	*81**	*83*	*93*
I10:SUM	*166*	*93**	*103*	*115*
I10:EX	*153*	*100*	*95*	*106*
I10:ENT	*147*	*104*	*91*	*102*
I10:ACT	*122*	*63**	*75*	*85*

*Tests for randomicity in time series. The number of observed turning points (third column) is compared to that expected for a random time series (95% confidence intervals; fourth and fifth columns; * indicates significant departure. Notes: Run numbers refer to those given in Table 3.3.2. Those prefixed I refer to independent measures in run LEPTO4 (I9) and LEPTO5 (I10); see text for details. N, number of independent observations*

3.4 Final Version of Image Analysis System

A revised version of the image analysis system was developed, which was utilized for all further image analysis described in later chapters of this thesis.

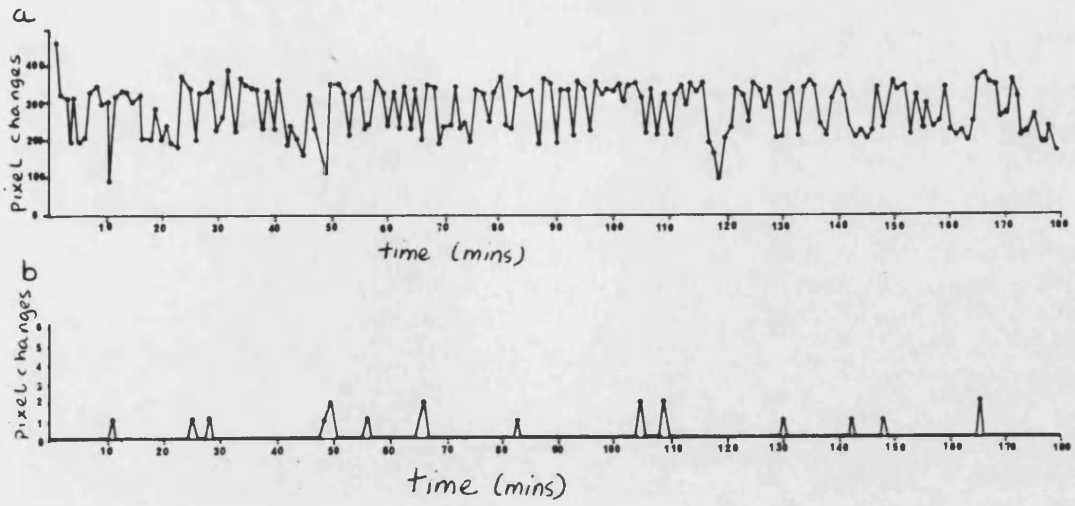

Figure 3.6: Time series of pixel mismatch counts for frames taken at 1 minute intervals for a stationary image. (a) Run 4 (Dead nest control), conducted before improved software to reduce noise. (b) Dead nest control measured after improvement in software.

3.4.1 Hardware.

The hardware finally arrived at was described in Section 3.2.1: colonies were lit from below by a X-ray viewing box, and filmed by a Fujinon TV zoom lens. This monochrome signal was fed directly to a Realitizer attached to the ROM port of an Atari ST1040 keyboard (Figure 3.3c). Data was stored on a 20 megabyte hard disc (Atari model SH204).

Experimental conditions.

All experiments were carried out in a constant temperature room regulated to 25°C and 50% humidity. Throughout the complete duration of any given set of experiments, the X-ray viewing box directly beneath the ant colony remained on (LL) permanently.

3.4.2 Software.

Framegrabbing software

The GFABasic program NC1.BAS developed by Tofts (1989) (see Appendix C.3) was utilized for frame grabbing. Like the program originally used (Sankson 1988), this allowed digitized frames taken at preset intervals to be stored to the hard disc. NC1.BAS was initially tested for its accuracy in timing over several 7 hour periods, and found to be accurate to within one second over this period (taking one frame per minute). Repartitioning of the hard disc allowed a maximum of 416 frames to be stored. After this point it was neccessary to analyse the stored frames before new frames were grabbed, a process that itself takes several hours. The constraints of memory and processing speed were therefore fundamental to the design of my experiments; the time taken to store a frame to memory when the hard disc was nearly full could reach the order of 30 seconds. Therefore although the efficiency of NC1.BAS would allow frames to be grabbed at 10 second intervals, the timing would rapidly loose accuracy as the hard disc filled; it would be unwise to take more than 300 frames at this interval and expect to maintain accurate timing. However, at 1 minute intervals, the time to store to disc could always be accomodated within the frame to frame interval, allowing the maximum number of frames to be stored, equating to nearly 7 hours of data. This regime (1 frame per minute, 416 frames) was employed throughout most of my experiments: 416 frames taken at a much longer interval would leave too little time overnight for their analysis before I required to grab more frames; 416 frames could not be grabbed at reliable time intervals if this interval was too short (below about 40 seconds). Also, one has to consider what trade- off to make between the resolution of data and the duration of data gathering (and hence the

number of events; activity cycles of the order of 20 minutes). As explained in Chapter 4, testing of mathematical models designed to account for the mechanism leading to the occurence of activity cycles neccessitated measurement of the gross characteristics of a large number of events (e.g., cycle length), rather than the detailed structure of fewer events. In any case, this system is not suitable for collection of fine resolution data on individual events (e.g., rates of change in activity levels over the short term), for reasons explained in Section 3.1: we cannot quantify the precise relationship between our measurements (number of pixel changes) and the parameter we wish to study (activity levels).

Analysis Software.

The frequent occurence of false positives in the control runs suggested that pixels were changing state under intensity fluctuations much smaller than those that occured when ants moved. Since it was not possible to adjust the hardware further to remove the occurence of such fluctuations, nor was it possible to alter the software provided by Print Technik to adjust the behaviour of the realitizer, a more subtle method of counting pixel changes had to be devised if the noise to signal ratio were to be reduced. In effect, the realitizer had too high a resolution for my purposes, and post frame grabbing image processing was required to "clean up" the image.

The software of Sankson (1988) was supposed to compare every tenth pixel (at "step 10"); however on further analysis, it was found to compare every tenth 8-bit string of pixels (i.e., a horizontal line of 8 pixels). Only if no pixels within such a string had changed state was no mismatch recorded. This counting technique will tend to overemphasize small changes between images, since the same result (one

mismatch) was recorded for any nonzero score of pixel changes out of 8. Since I wished to de-emphasize very small changes between images, this algorithm was clearly not appropriate.

Skeletonization, a class of standard algorithms for noise filtration in images, was also unsuitable (C. Tofts, pers. comm.). Skeletonization is applied to individual images: the boundary between (black) objects and (white) background is "cleaned" by changing focal black pixels to white, if a certain number of neighbouring pixels are white. The procedure may be iterated a number of times to further reduce "fuzzy edges". Such a technique would remove isolated black pixels (noise) from the background, and in theory could reduce each ant to a single line. However, since the image quality from my system was such that individual ants were sometimes represented by two or more small areas of black separated by white "background" (see Figure 3.1) for example, due to reflection of light off shiny areas of cuticle, skeletonization may also have reduced signal by removing some ants from the picture. Similarly, skeletonizing the "fuzzy edges" of the brood pile may have reduced the signal, since activity here is measured largely as a result of changes in the overall shape of the brood pile; that is, changes at the interface between this dense black area and the background.

A program utilizing "false colouring" was developed by C. Tofts (AMW1.C; see Appendix C.4). Pixels are grouped into blocks or squares. For my purpose, a square of 4 $\times$ 4 pixels was chosen, representing about the area occupied by one ant head on the screen. Blocks are assigned a "colour" on the basis of the number of black pixels that they contain; I shall refer to these colours as "white", "black", and "grey". Colour is determined on the basis of two thresholds: if the number of black pixels is below the lower threshold, the block is "white"(Figure 3.7); if there are more black pixels than the upper threshold, the block is "black". If

the number of black pixels lies between these two thresholds, the colour "grey" is assigned. Consecutive images are converted to this form and the individual blocks representing the same portion of the screen are compared. A mismatch is scored only if a "black" block has changed to "white", or *vice versa*. No mismatches are scored for any other transition, i.e., any transition involving "grey" blocks. By adjusting the values of the lower and upper thresholds it is possible to obtain various noise to signal ratios; as the band of values falling between the two thresholds is increased, so is the amount of "movement" required to register as a mismatch. However, if the grey band accounts for too large a proportion of the possible spectrum, no change at all (including gross ant movements) will be registered. I therefore callibrated the system for a number of different block sizes. Using the final hardware design outlined in Section 3.4.1 above, images of a dead nest control were taken at appropriate magnification. Analysis of the frames was rerun for different threshold values to optimize the trade off between minimum noise and maximal signal response. Thresholds were adjusted to achieve mismatch counts no greater than 2 per frame comparison, whilst minimizing the value (upper threshold−lower threshold) that is, minimizing the length of "grey band". The results of this cal ibration are presented in Table 3.4.1; for a block size of 4 × 4 pixels, a lower threshold of 8 and an upper threshold of 11 was chosen.

Table 3.4.1

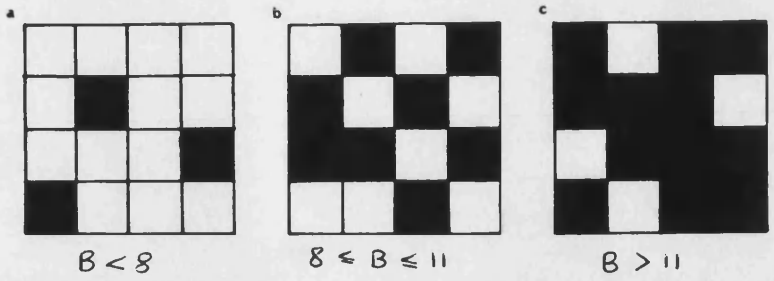

Figure 3.7: Assignment of states to blocks of pixels:"false colouring technique". Pixels are formed into groups of 16, called blocks. The state of the block is decided on the basis of the number of 'black' pixels (B) within a block. A mismatch is counted if a block changes from white to black or *vice versa.*

Block Size	*L*	*U*	*r*	*Pixel Changes*
10	*25*	*45*	*3*	*1*
9	*18*	*41*	*3*	*1*
8	*16*	*36*	*5*	*1*
7	*14*	*33*	*3*	*1*
6	*12*	*28*	*3*	*1*
5	*5*	*23*	*3*	*1*
4	*7*	*13*	*1*	*1*
4	*7*	*12*	*2*	*1*
4	*8*	*12*	*3*	*2*
4	*8*	*11*	*5*	*2*
4	*9*	*11*	*2*	*20*

Cal ibration results for false colouring technique. The number of pixel mismatches found on analysis of a dead ant nest control for various upper(U) and lower(L) thresholds to block state determination is given. Notes: block size indicates dimension; hence block size 4 implies a 4 × 4 block (16 pixels). Thresholds: for explanation see text, grey is assigned to block when $L \leq x \leq U$ *where x is the number of pixel changes between frame within a given block. r is the number of replicates; analysis under same settings of different control run. Pixel changes; the maximum number observed over all replicates (for a single frame comparison).*

When the image is analysed as blocks each consisting of 16 pixels, a maximum of 2000 pixel mismatches per frame comparison may be registered. The number

of pixel changes recorded from frames of a live ant nest tended to lie between 100 and 500 (see Chapter 4). Therefore the noise to signal ratio under the above criteria can be estimated at worst to be 0.02 (noise as false positives only).

Since this program processes all the information within each frame (unlike the step function of Sankson's 1988 program), it was encoded in the C language rather than Basic to improve running speed. The program also enabled activity within portions of the nest to be monitored, by designating "windows" in which pixel changes were counted separately. This feature allowed the analysis of activity patterns in various areas within and outside the nest, as explained in the next chapter. Running time when 15 such windows were specified was roughly 1 minute per frame comparison, hence this program required 7 hours to analyse the maximum number of data files that could be stored to hard disc.

Chapter 4

Activity Patterns Within Ant Nests

4.1 Introduction

Several studies have indicated the existence of short term cycles of activity in nests of *L. acervorum* (Franks and Bryant, 1987; Franks et al., 1990a) and more recently, in the Myrafant *L. allardycei* (Cole, 1991a). These papers report that cycles arise at the colony level as a result of individuals synchronizing their activity. The cycles, variously described as rhythmic, periodic or pulsatile, are in the order of 20 minutes for *L. acervorum* and 35 minutes for *L. allardycei.*

A number of models have been proposed to indicate the mechanism (Goss and Deneubourg, 1988; Tofts, 1990a) and function (Hemerik et al., 1990) of these cycles in colony activity. Previously, data concerning this phenomenon has been collected manually (Franks and Bryant, 1987; Franks et al., 1990a) although Cole (1991a) has used automated techniques similar to those outlined in this thesis. The reliance on time consuming manual techniques has precluded a precise

description of activity patterns, or a test of the various models.

My aim in this chapter is to provide a description of activity patterns in laboratory nests of *L. acervorum* and in the Myrafant *L. tubero-interruptus* as a comparison, and test the models of Goss and Deneubourg (1988), Hemerik et al. (1990), and Tofts (1990a) from data collected using the image analysis system developed in Chapter 3. I discuss the models with reference to their assumptions and predictions. Further comparison of a technical nature is presented in Tofts et al. (1992).

Simulation

Goss and Deneubourg (1988) present a model based on autocatalytic interactions between ants, in which active ants cause others to become active by disturbing them. Individual ants are assumed to behave as follows:

- once an ant becomes inactive it will remain so for some fixed period, after which it becomes 'activatable';
- at each instant, activatable ants may become active spontaneously with some fixed probability;
- active ants move at random, causing any activatable ant they happen to encounter to become active;
- at each instant, active ants may become inactive with some fixed probability.

Hence positive feedback between active and activatable ants serves to synchronize bouts of activity at the colony level. A lag period during which ants do not respond to stimulus from others or wake spontaneously results in phases of inactivity. This model was presented as a computer simulation, in which 50 ants that obeyed the above constraints were simulated using Monte Carlo methods. A sample result was presented as a time series showing per cent colony activity at arbitrary time intervals. The time series exhibited pulses of activity, rising rapidly and decaying more slowly, interspersed with intervals of inactivity. The sample shown suggested that synchronization was only partial, no more than 60% of ants being active at peak times. Goss and Deneubourg describe the bursts of activity as regular, the period of the cycle being roughly equal to that of the inactive phase of individuals. They also reported that "small" collections of ants generated nonperiodic activity patterns.

This model yielded few testable predictions, apart from qualitative comparison of cycle shape to that generated by the model, and that small colonies should not exhibit periodic activity. Analysis of predicted cycle length distribution is not presented, and measurement of cycle length itself does not allow refutation of the model since parameters such as inactive phase and probability of becoming active or inactive are left free. Qualitative comparison of cycle shape using data collected by automated techniques is not possible, due to nonlinear relationship between the number of pixel mismatches and the number of active ants.

Activity Linked to Energy Level

Hemerik et al. (1990) present a model in which fluctuations in colony energy level drive the cycles of activity. The rate of increase of energy (E) stored in the

nest is assumed to be proportional to the number of active ants (A; a subset of which are successful foragers, denoted by the constant a) minus a term for energy depletion:

$$\frac{dE}{dt} = aA - bE \qquad \text{(i)}$$

The rate of increase in the number of active ants is assumed to be inversely related to energy level. Further, rate of change in A is assumed to be a quadratic function of A to allow a rhythmic solution to the system of equations:

$$\frac{dA}{dt} = \frac{c}{d+E}(eA^2 + fNA + N^2)(N - A) - gA \qquad \text{(ii)}$$

Where N is the total number of ants, and all the constants $(a - g)$ are strictly positive.

Hemerik et al. (1990) relate increase in activity to decrease in energy (equation (ii)) to incorporate an adaptive perspective to the model; hence stating "low levels must stimulate activity to ensure colony survival". For certain values of the constants, the equations exhibit cyclic behaviour in the number of active ants.

The testable predictions of this model are a consequence of the relationship between the period of the limit cycle solution and relative rates of energy change. For colonies with higher brood to worker ratio (larger b), they predict a decrease in cycle length and eventual breakdown in cycles altogether above some threshold of b. If the colony undergoes starvation (a tends to 0), ants should reduce their resting period (from the assumption of inverse relationship between A and E), hence increasing cycle frequency. Also, as starvation is prolonged cycles should

breakdown as A tends to a constant high value. Hence Hemerik et al. (1990) model can be tested by measurement of the relationship between cycle length and brood to worker ratio or starvation.

Algebraic Description

Tofts (1990a) presents a simplified version of the autocatalytic system of Goss and Deneubourg (1988); the terms wake, wakeful and sleep are used to denote active, activatable and passive phases:

- an ant will sleep for a determined period;
- it will then become wakeable at which time it may wake up with some probability at each subsequent instant;
- if it wakes up it will wake any other wakeful ant;
- having woken up it will then fall asleep again.

These assumptions, and the relationship of this model to that of Goss and Deneubourg (1988) are discussed further in Section 4.5. The above description of an ant was encoded in a particular form of process algebra (Milner, 1983, 1989; Hoare, 1985; Beaten et al., 1986); the weighted synchronous calculus of communicating systems (WSCCS; Tofts, 1990b). Such process algebras are formal description languages used mainly in the field of computer science to allow effficient reasoning about concurrent systems such as electronic mail, concurrent operating systems and communications protocols. However, an ant colony is also a concurrent system; the behaviour of the system results from the actions and interactions of many functional units (ants).

The process algebra description of an ant and a colony is given in Tofts (1990a) and Tofts et al. (1992). Tofts (1990a) proves that a collection of ants obeying the above criteria will synchronize their activity, producing cyclical colony activity, and that the synchronized state is stable. He also shows that the frequency of activity cycles will be independent of colony size, for colonies larger than *circa* 30 ants. The model also predicts that cycle length will be distributed as geometric decay, commencing after a fixed lag s, equivalent to the length of sleep phase of an individual. This distribution results from the probabilistic waking of ants after s is exceeded. The model was further refined to incorporate a variable sleep phase (Tofts, 1990a); this version is discussed in Section 5.4. Hence the model of Tofts (1990a) can be tested by measurement of the relationship between cycle length and colony size, and by measurement of cycle length distribution.

4.2 Methods

Six experimental runs, each requiring roughly 3 weeks for completion, were implemented on different nests (4 colonies of *L. acervorum*, and 2 colonies of *L. tubero-interruptus* for comparison); run dates and colony characterizations are presented in Table 4.2.1. Each experimental run was implemented as follows: 48 hours prior to the start of data collection, the petri dish containing a colony housed in a nest of dimensions 60 × 35 × 2 mm as described in Chapter 2 was placed on top of an X-ray viewing box in the constant temperature room, as described in Sections 3.2.1 and 3.1. This allowed the colony 48 hours to acclimatize to conditions in the constant temperature room. The colony was provided with tap water and 1 M sucrose solution in cotton wool plugged plastic tubes, and *Drosophila* larvae were also placed in the petri dish during the acclimatization period.

Table 4.2.1

Run	*Start Date*	*D*	*W*	*Q*	*Eggs*	*S*	*Me*	*L*	*P*	*M*
BIG+	*19/7/90*	*16*	*216*	*3*	*148*	*62*	*60*	*58*	*11*	*0*
MID+	*3/9/90*	*16*	*56*	*2*	*12*	*22*	*56*	*8*	*0*	*0*
LIT+	*24/9/90*	*16*	*46*	*6*	*0*	*57*	*12*	*15*	*1*	*0*
SMA+	*16/10/90*	*15*	*12(23)*	*1*	*20(5)*	*13(15)*	*7(2)*	*4(3)*	*12(0)*	*0*
*DIF**	*12/11/90*	*17*	*53*	*0*	*26*	*16*	*14*	*3*	*3*	*0*
*TUB**	*19/10/91*	*16*	*135*	*1*	*23*	*54*	*27*	*15*	*0*	*0*

*Summary of image analysis experiments introduced in Chapter 4. Start date refers to the date on which first digitized data collected. + denotes L. acervorum, * denotes L. tubero-interruptus. SMARUN: colony charateristics changed during the run, figures in parentheses denote charactersitics at the end of the run. D: number of days of digitized data collection; other columns refer to the number of items as characterized in Chapter 2: W, workers; Q, queens; S, Me, L, small medium and large larvae; M, males.*

Data was gathered as summarized in Tables 4.2.2 a, b; the daily run commenced at 11 am GMT, when NC1.BAS was set to run. This program directed storage of one digitized frame per minute for 416 frames. After the program had finished the program AMW1.PRG was set running to analyse the binary images. A block size of 4 × 4 pixels was utilized for false colouring with a lower threshold of 8 and an upper threshold of 11 (see Section 3.4.2). In addition to analysing the complete image, 9 separate "windows" were also analysed; the size and position of windows was set by altering a parameter file loaded into the program. The windows set for these runs are depicted in Figure 4.1; see also Table 4.2.3.

Table 4.2.2

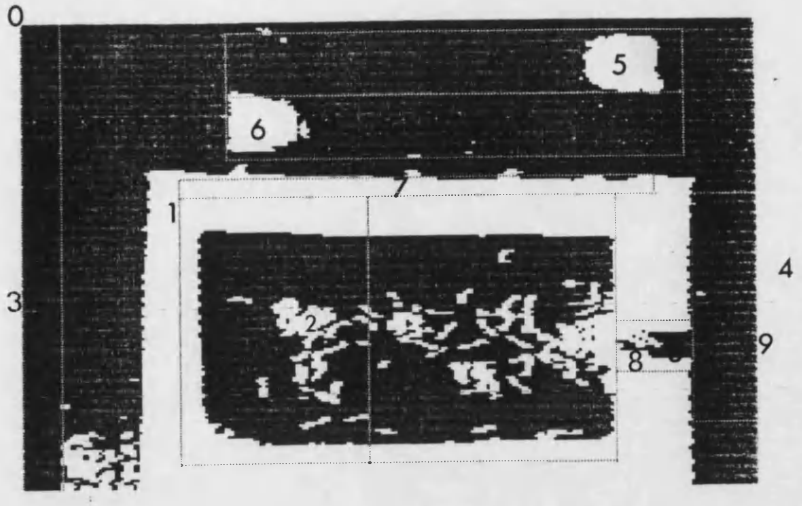

Figure 4.1: Windows for which separate image analysis data was collected. 0: whole field of view. 1, inside nest; 2, Brood Pile; 3, Left edge; 4, Right edge (3,4 outside petri dish); 5, forage tube; 6, water tube; 7, control; 8, nest entrance; 9, run. 3 and 4 are subtracted from 0 to obtain whole petri dish.

a

Number of days	*16*
Duration per day	*7 Hours*
Frames per day	*416*
Start	*11am GMT*
Frame Frequency	*1 per minute*
Progam used	*NC1. BAS*

b

Program employed	*AMW1.PRG*
Windows per frame	*9*
False Colouring:	
Block size	*4 × 4*
Lower Threshold	*8*
Upper Threshold	*11*

Summary of protocols for image analysis. (a) frame grabbing procedure; (b) analysis of frames to detect pixel mismatches.

Table 4.2.3

Window	*Area Covered*
0	*Whole field of view*
1	*Nest*
2	*Brood Pile*
3	*Edge to left of petri dish*
4	*Edge to right of petri dish*
5	*Sugar tube*
6	*Water tube*
7	*Control*
8	*Nest entrance*
9	*"Run" by entrance*
X0	*0-3-4: Whole petri dish*
X1	*1-2: Nest minus brood pile*
X2	*X0-1: Outside nest*
X3	*8 + 9: Entrance plus run*
X4	*X1 + X3: "door hangers"*

Key to windows within which analysis of activity levels was performed. The position of logical partitions forming windows was set within the program AMW1.PRG; post gathering analysis was performed on Windows 0 to 9. Activity within windows prefixed X was obtained by appropriate manipulation of pixel mismatch scores in windows 0 to 9.

The colony itself was inspected after the completion of daily frame grabbing, so as to minimize disturbance. Water and sucrose were replaced when neccessary (roughly every 5 days); if this occurred, *Drosophila* larvae were also added.

Thus maintenance of the experimental run required two visits daily to the constant temperature room. Data collection itself and initial analysis was entirely automated. Data was collected on consecutive days whenever possible, to minimize the time spent by any colony in the rather extreme conditions of the constant temperature room (*vis a vis* light in particular), and to mantain minimum variation in disturbance. An earlier test run over 3 weeks using this protocol except that data was not gathered at weekends, resulted in higher levels of pixel

changes (and therefore activity) for data sets gathered on each of 3 Mondays. Although this "experiment" was not repeated, the most obvious interpretation of this observation was that renewed human activity after a weekend break resulted in increased disturbance of the colony, presumably the result of vibrations from walking and shutting the heavy door. Inevitably, some variation in disturbance levels did occur, since other researchers also required access to the room. Also, some breaks in data collection on consecutive days were neccessary, due to hard disc failure, failure of constant temperature regulation, and electrical maintenance. Such breaks were recorded by appropriate naming of data files.

In most cases, data were gathered for 16 days, a compromise between rate of turnover of experiments and the need to accumulate sufficient samples of cycle length for goodness of fit tests to model distributions (see Section 4.3.6). In all cases, experiments continued beyond the point at which the 95% confidence interval on cycle length estimation was exceeded by the known error in measurement inherent in the system (+/- 1 minute, for frames taken at 1 minute intervals).

On the day following the last day of digitized data collection, the colony was filmed from 11 am to 6 pm by attaching the camera utilized for digitization to a Panasonic NV-8050 timelapse video recorder. Film was recorded at 16× natural speed on TDK HS E-180 VHS video cassettes. This film was analysed as described in Chapter 5. After completion of filming, the colony was replaced in the laboratory, and the numbers of brood and adults counted. This characterization was made after the experimental run, as it may have added to disturbance.

4.3 Results (Descriptive)

4.3.1 Time Series

The raw data from daily sessions lasting 7 hours can be presented as a time series of colony activity (measured as number of pixel changes between successive frames). Figure 4.2 a presents one such time series for activity within the whole petri dish (window 0; windows 3 and 4 subtracted - see Table 4.2.3) for day 4 of the run MIDRUN. For data in this chapter alone, at least 1000 such graphs could be presented, consisting of 97 daily records of activity within the whole petri dish, and a further 97 for activity within each window of interest, including portions of the nest not measured directly. For example, we can obtain the activity level outside the nest by subtracting that within the nest from that in the whole petri dish. I do not feel that it would be particularly instructive to present all this data; and hence have presented a sample only from the various runs, for various windows, in Figure 4.3 and Appendices D.1 and D.2.

Raw data activity time series for the complete field of view (nest and surrounding petri dish) depicted in Figure 4.2a and Appendix D.1 suggest that colony activity level oscillates in a cyclical manner, but with considerable variation at short time intervals. Activity does not appear to fall to zero; the maximum number of pixel changes rarely exceed 3 times the minimum. I will return to this in Section 4.5.1. Cycle length appears to be roughly 20 minutes, but varies considerably (compare Figure 4.3 a, where 6 cycles occur in 1 hour to Figure 4.3 b, where there are 1-2 per hour). Cycle length in nests of *L. tubero-interruptus* (for example Figure 4.3 c) appears to be longer (in the order of 30 minutes). There is some suggestion that time series for this species show less short term fluctuations, with more

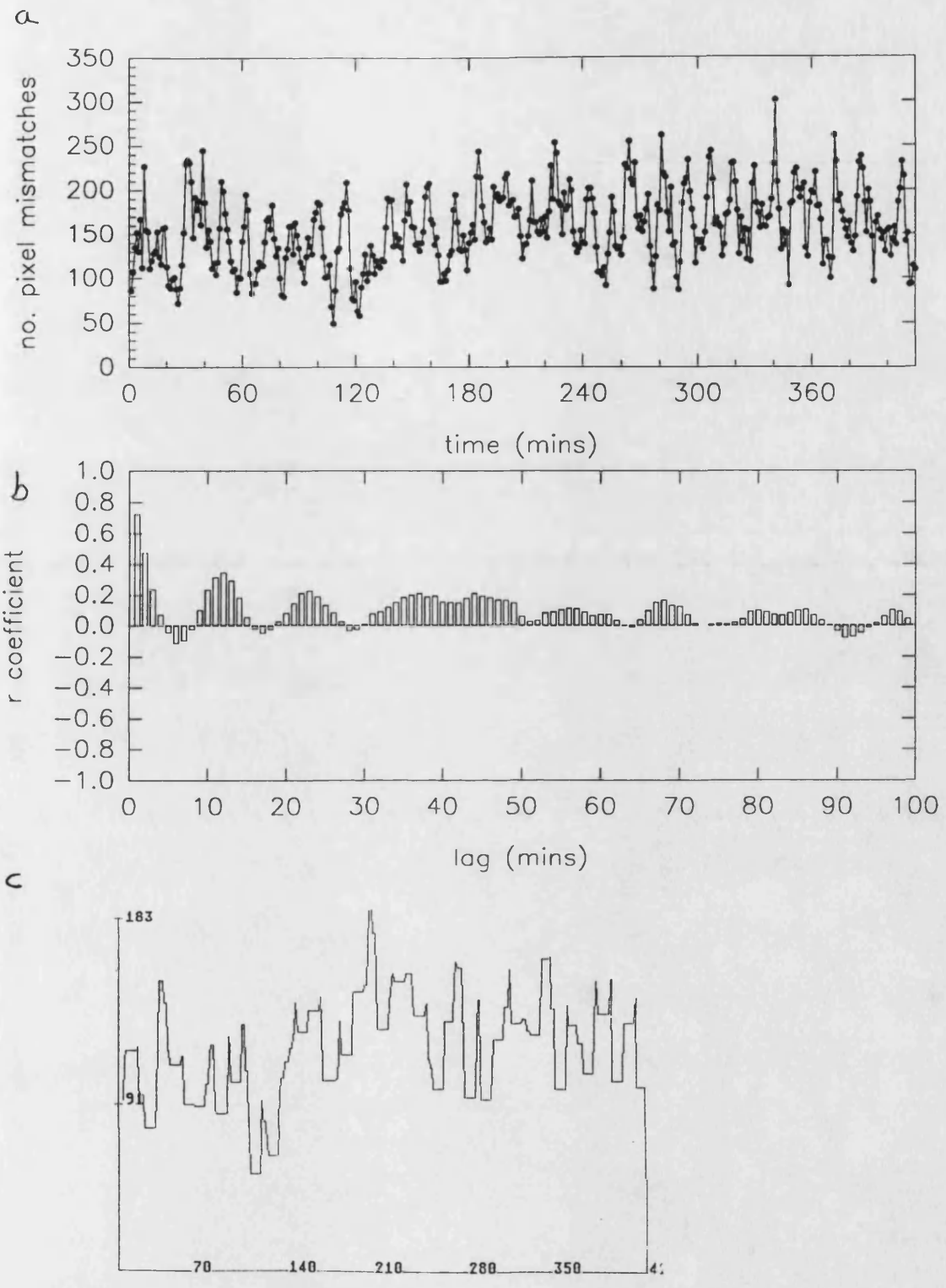

Figure 4.2: Sample daily output: time series for day 04 of MIDRUN, window X0. (a) raw data time series; (b) autocorrellogram of (a); (c) result of moving minima procedure applied to (a), for explanation see text.

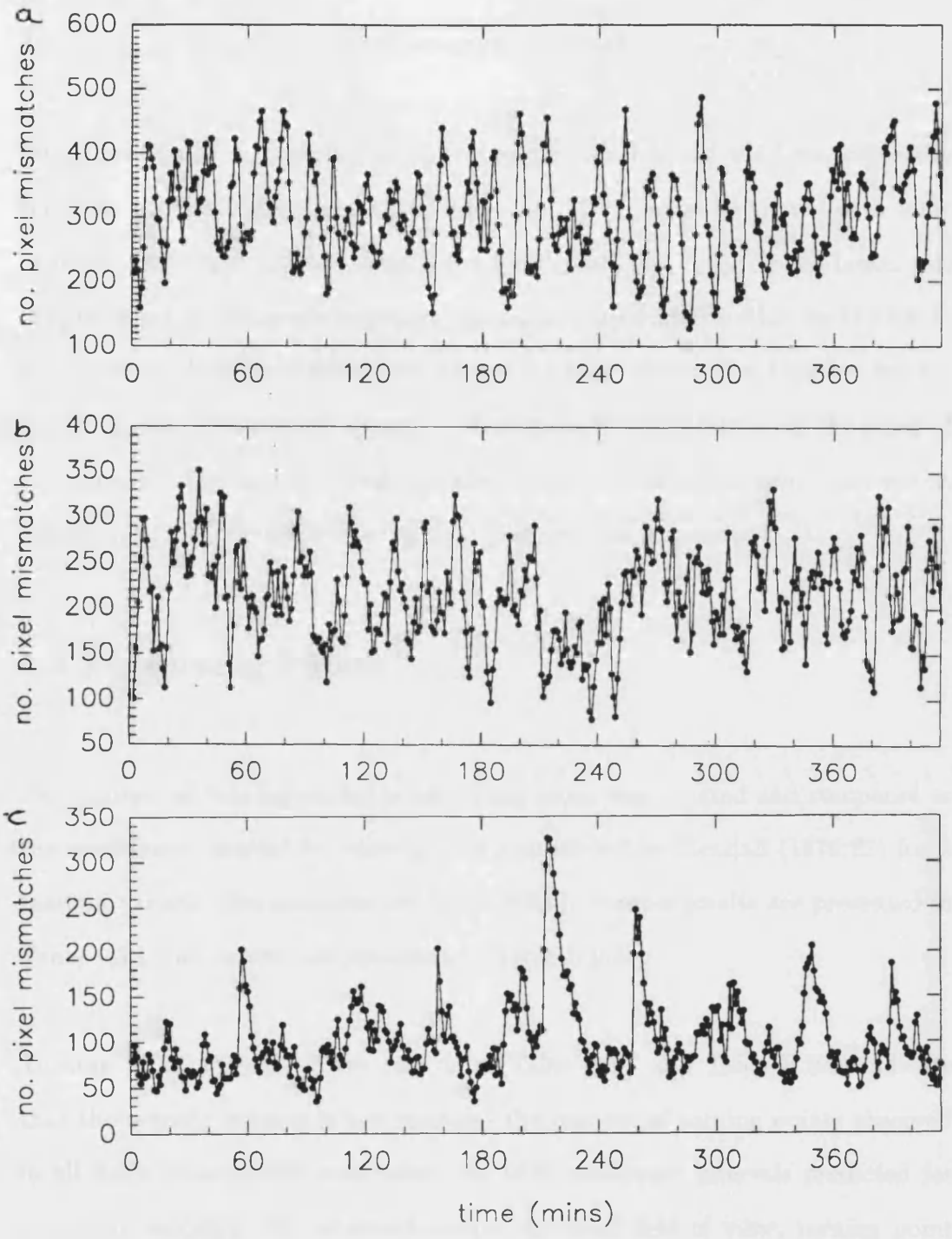

Figure 4.3: Sample activity time series for whole petri dish (window X0). Run identity is indicated by the run name and following day number. (a) BIGRUN17, exhibiting cycles at high frequency and beating phenomenon (*L. acervorum*); (b) LITRUN05, exhibiting longer cycles (*L. acervorum*); (c) TUBRUN04, showing long cycles with marked peaks (*L. tubero-interruptus*).

distinct sharp peaks in activity between longer bouts of inactivity.

Interpretation of the number of pixel changes in individual windows, especially those for which the ratio of perimeter to area is high, must be viewed with some caution. Raw data activity time series for the window covering the brood pile (Figure 4.4a), nevertheless indicates cyclical activity similar to that for the whole field of view. Activity level within window X1 (nest-brood pile; Figure 4.4b) are less obviously cyclical, and appear to show more frequent bursts (of the order of 11 minutes). The activity levels in other windows (entrance, run, food, water, outside nest) are not obviously cyclical (Figure 4.4c; Appendix D.2).

4.3.2 Turning Points

The number of turning points in each time series was counted and compared to the confidence interval for turning points predicted by Kendall (1976:22) for a random variable (for methods see Table A.0.6). Sample results are presented in Table 4.3.1; full results are presented in Table B.0.3.

Turning point analysis of the raw data (Table 4.3.1 and Table B.0.3) indicates that the activity pattern is not random: the number of turning points observed in all daily sessions falls well below the 95% confidence intervals predicted for a random variable. For windows within the total field of view, turning point analysis (Table B.0.4) generally indicates that the activity time series in most windows is not random.

Table 4.3.1

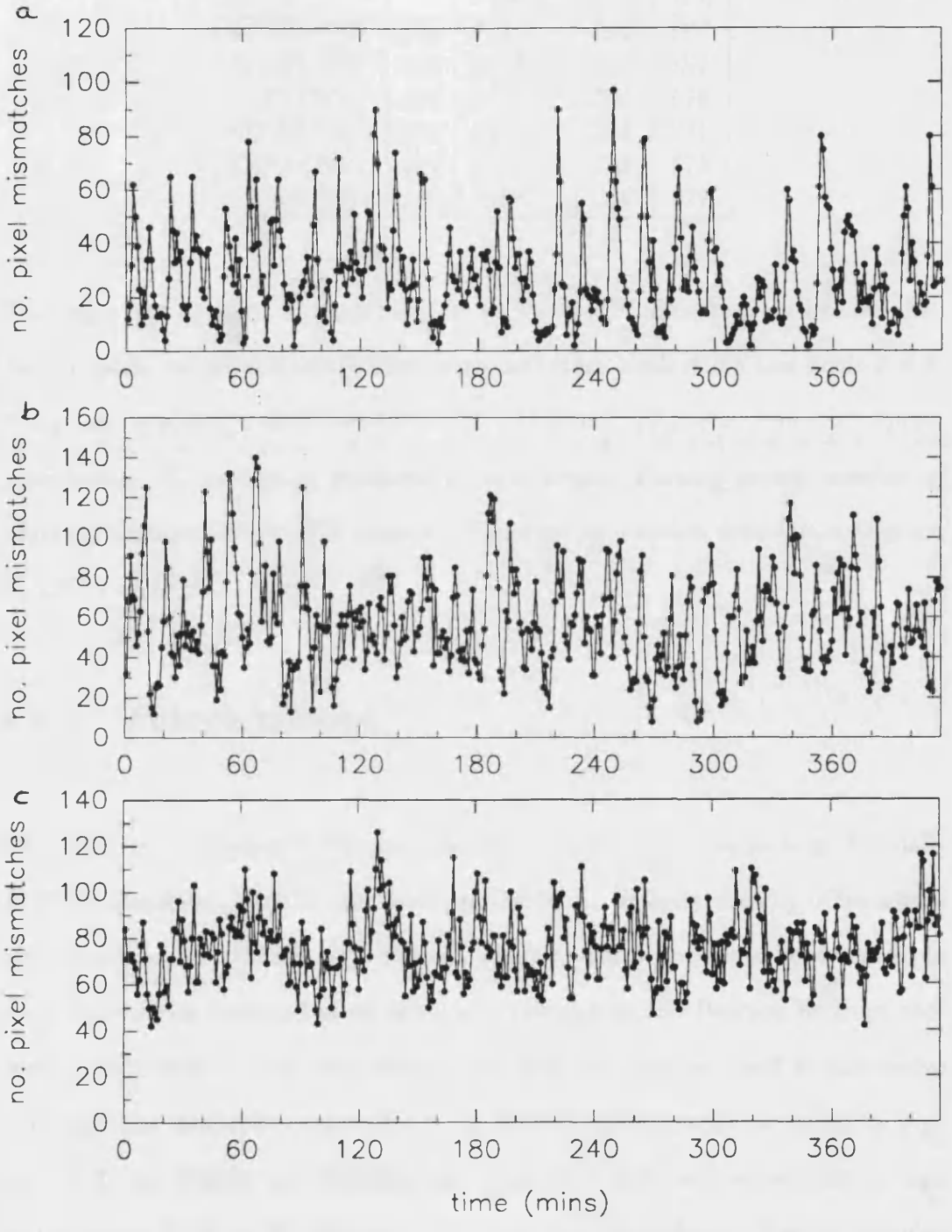

Figure 4.4: Activity time series for windows within the field of view. Data from day 10 of MIDRUN is presented. a, window 2 (brood pile); b, window X1 (nest-brood pile); c, window X2 (outside the nest).

Run Day	*N*	*Turning Points*	*LCI 95%*	*UCI 95%*
BIGRUN03	*413*	*211**	*265*	*283*
MIDRUN01	*411*	*200**	*264*	*282*
LITRUN01	*405*	*202**	*260*	*278*
SMARUN01	*410*	*226**	*263*	*281*
DIFRUN01	*404*	*211**	*259*	*277*
TUBRUN01	*404*	*199**	*259*	*277*

*Turning point analysis of daily time series (samples). Results from window X0. For complete results and explanation of procedure see Table A.0.6 and Table B.0.3. * indicates significant departure (P< 0.05) of turning point score from the random expectation. N, number of independent data points; Turning points, number of observed turning points; 95% confidence interval for random expectation is given by LCI and UCI.*

4.3.3 Autocorrelation

This standard technique for the analysis of periodicity in time series (e.g., Kendall, 1976:87; Chatfield, 1984:23) has been applied to ant activity data by other workers (Franks et al., 1990a; Cole 1991a). I performed autocorrelation for sample daily time series (conducted on Minitab), calculating the Pearson product moment coefficients for the time series data correlated against itself at successive time lags (for details see Appendix A.1). Sample correlograms are shown in Figure 4.2 b and Figure 4.5, showing significant (P< 0.05) self correlation at lags in the range of 10 to 30 minutes. Generally, the correlograms (further samples presented in Appendix D.3) tend to indicate cyclicity at periods considerably shorter than 20 minutes. Frequently the first (minimum) negative correlation occurs at a lag of 6 to 7 minutes (indicating a half period) and the first (maximum) positive correlation occurs at time lags in the range of 12 to 16 minutes

(indicating one period length); see Figure 4.2. The correlograms are frequently cyclical in form and do not decay rapidly, again indicating cyclicity in the raw data (Chatfield, 1984:28). Correlations at longer time intervals generally appear to be harmonics (the lag is a multiple of the first lag with significant correlation of the same sign). For reasons discussed in Section 4.5.2 (see also Enright (1981)), autocorrelation cannot be used to provide estimates of mean cycle duration in this system. Hence autocorrelation was employed only to confirm or reject statistically the subjective interpretation that time series were "periodic". In conclusion, autocorrelation indicates a tendency towards cyclicity, but with considerable variation between cases. On the basis of this analysis cycle length does not appear to be fixed, resulting in different estimates of period on different days, and in correlograms without strong indications of cyclicity on some days.

4.3.4 Other Evidence For Cyclicity

Further evidence for cyclicity in the time series data is provided by the shape of first return maps (for example, Figure 4.6 a for the whole field of view). When the activity level at time t is plotted against that at time $t + 1$, a cyclical graph is produced. The shape of the graph above appears to be characteristic of all samples examined; that is, a circle flattened in the leading diagonal axis. The diameter of return cycles (measured on the leading diagonal) shows considerable variation between cycles, indicating that the amplitude of activity varies between cycles. The number of points forming a return cycle is also quite variable; indicating that cycle length is not fixed. The precise shape of return cycles tends to suggest that cycles consist of a relatively fast rise in activity (few points on leading edges of return maps) followed by a slow decline in activity (more data points on the return edge). Dense clustering of return map points near the ori-

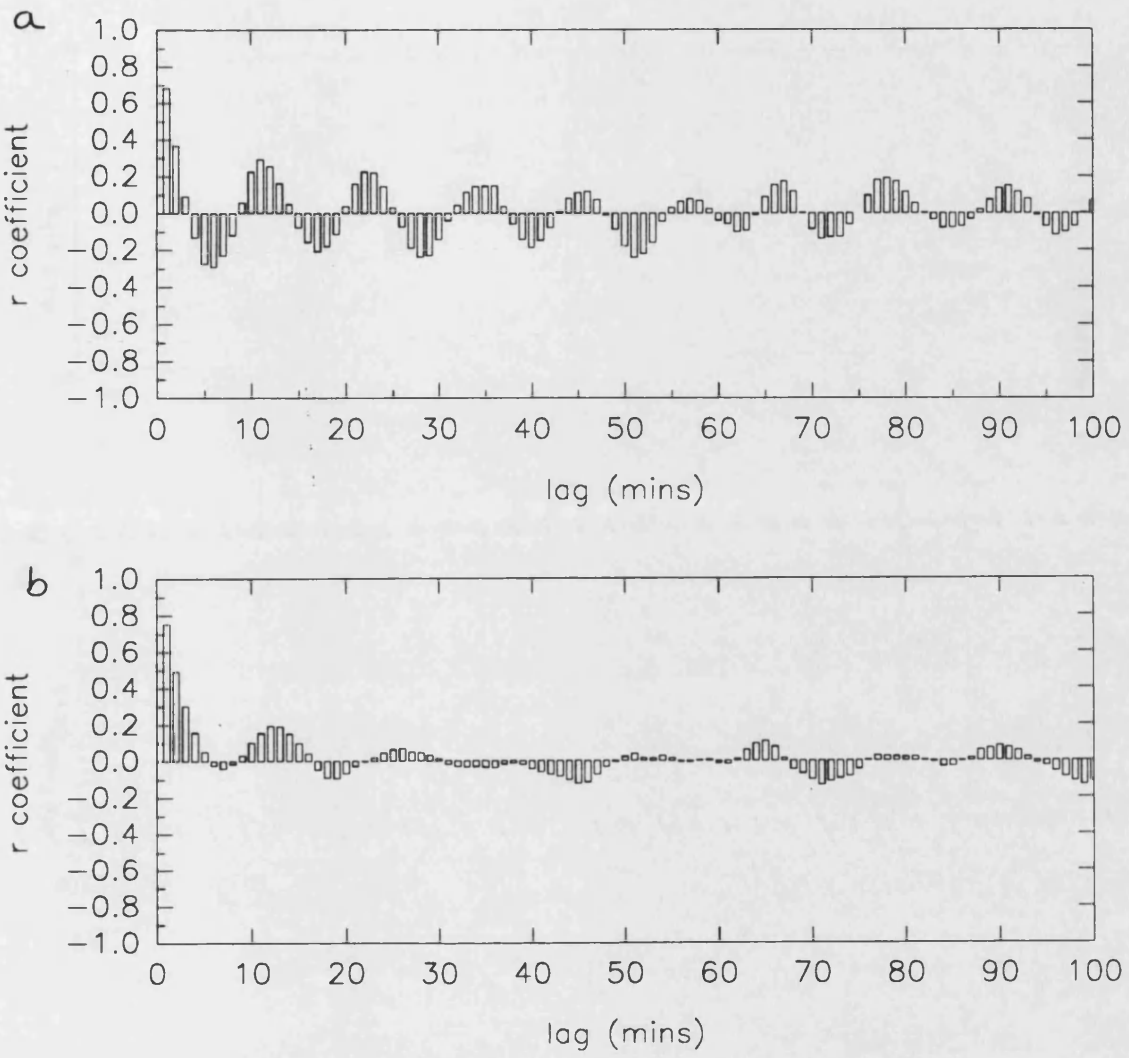

Figure 4.5: Sample correlograms from activity time series (window X0). a, BIGRUN04; b, LITRUN05.

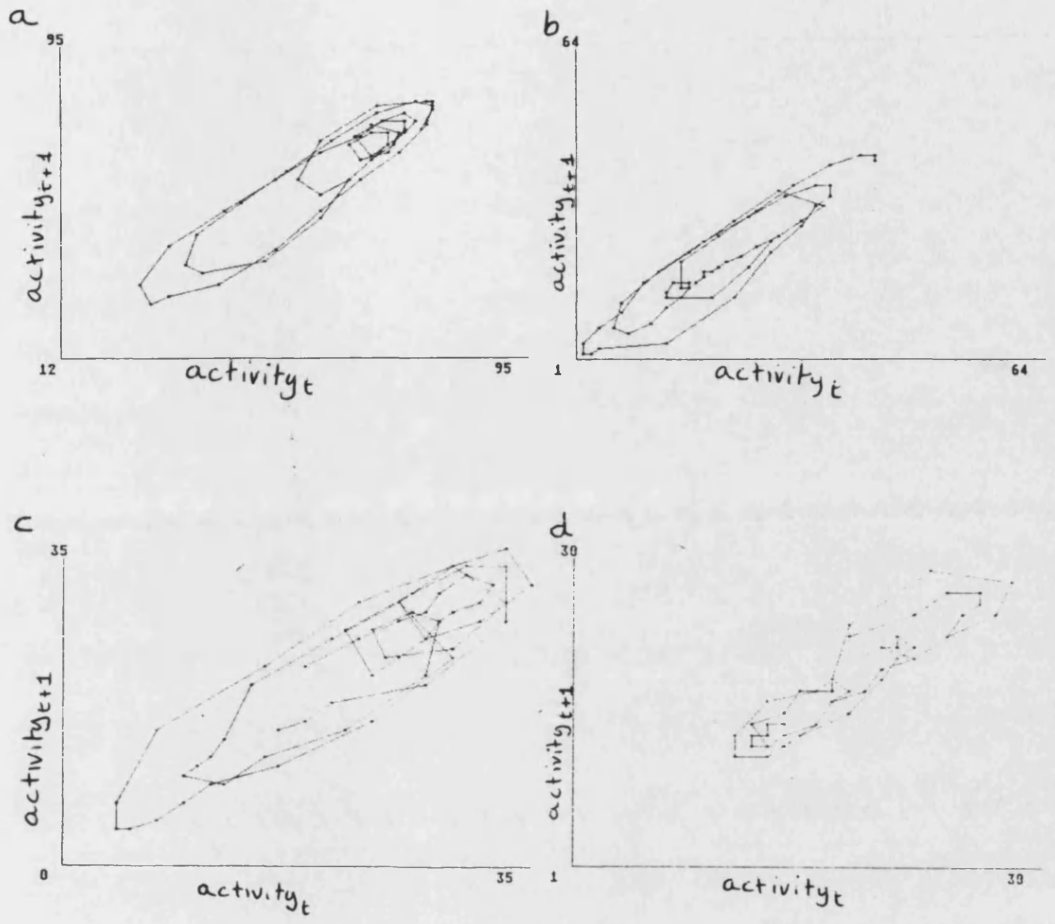

Figure 4.6: Sample return maps of activity for day 01 of SMARUN. In each graph, activity at time t (horizontal axis) is plotted against activity at $t + 1$ (t in one minute intervals). a, Window X0; b, window 2; c, window X1; d, window X2.

gin and relatively few points at high activity levels also suggests that activity patterns consist of relatively long periods of low activity interspersed by short bursts of high activity. The return map of activity on the brood pile (for sample see Figure 4.6 b) indicates cyclicity with the characteristics mentioned above. There is some suggestion (closer similarity between return cycles) that the brood pile cycles have more regularity. Further supporting visual interpretation of time series, the return map of activity in window X1 (nest-brood pile; Figure 4.6 c) exhibits greater variability in return cycle diameter, and individual cycles tend to contain fewer points. The return map for window X2 (outside nest; Figure 4.6 d) does not appear to exhibit cyclical behaviour. Hence, it appears that activity outside the nest does not follow a cyclical pattern, whereas activity within the nest may vary cyclically. Further, there is some evidence to suggest that activity on the brood pile exhibits more regular slower cycles than that away from the brood pile.

4.3.5 Cycle Length Determination

Due to the nonlinear relationship between pixel changes and colony activity as discussed in Chapter 3, it is difficult to interpret the precise meaning of peaks in the time series data. However, we can be reasonably certain that troughs (low number of pixel changes) equate to low levels of activity, as the processing of binary image data has reduced the probability of false positive occurence below P=0.05. Hence, in order to locate cycles, points of minimum activity were used as markers. Using the GFABasic program SPREDLOC.BAS (see Appendix C.2) raw data were viewed as a timeseries graph on the screen of an Atari ST1040 computer. The data were processed through a moving minima procedure employed to make local minima clearer. I then marked each local minimum by depressing a

mouse button, and the resulting cycle lengths were output to a file. The moving minima procedure (so called owing to its resemblance to moving average routines) is presented in Appendix A.2. The results of this moving minima procedure are shown in Figure 4.2 c.

Files of cycle length from daily sessions were fed to a statistical program, ASTATA.BAS (Appendix C.1). This program calculates mean and variance of cycle length for daily sessions and complete runs. It also calculated the goodness of fit of cycle length distribution to the distribution predicted by the model of Tofts (1990a), as described in the next section.

Table 4.3.2 summarizes the mean and variance of cycle length for the six runs. The mean cycle duration for all 4 runs involving *L. acervorum* are notably similar, being 1239, 1307 1326 and 1305 seconds. None are significantly different on the basis of 95% confidence interval overlap. The means of cycle duration for *L. tubero-interruptus* are significantly different (1470 and 1642 seconds), both from each other and from those of *L. acervorum.*

Table 4.3.2

Run	*N*	*Mean Cycle Length*	*Variance*	*LCI 95%*	*UCI 95%*
BIGRUN	*293*	*1239*	*106539*	*1201.63*	*1276.37*
MIDRUN	*270*	*1307*	*69160*	*1275.63*	*1338.37*
SMARUN	*263*	*1305*	*104427*	*1265.94*	*1344.06*
LITRUN	*277*	*1326*	*79609*	*1292.77*	*1359.23*
DIFRUN	*265*	*1470*	*116482*	*1428.91*	*1511.09*
TUBRUN	*218*	*1642*	*208247*	*1581.42*	*1702.58*

Summary of cycle length statistics. Cycle lengths (in seconds) were measured by the trough location procedure applied to activity time series from window X0. For

N cycles observed, mean cycle length is given in seconds; 95% confidence intervals (calculated from Students T) are given by LCI and UCI.

4.3.6 Cycle Length Distribution

Skewness and Kurtosis

Using the measurements of cycle length collected by the trough location procedure outlined in Appendix A.2, frequency distributions of cycle length for each run were compiled. These are presented in Figure 4.7; for the four *L. acervorum* runs the distributions clearly deviate from a normal. The modal cycle length is close to (but not equal to) the observed minimum cycle duration, and is of the order 1300 seconds. The minimum itself is clearly non zero in all cases, indicating that cycle length is at least 600 seconds for *L. acervorum* and 700 seconds for *L. tubero-interruptus.*

The deviation of these distributions from a normal distribution was investigated by calculating both third and fourth moments (g_1 and g_2) measuring skewness and kurtosis (Sokal and Rohlf, 1981:114).

The statistics g_1 and g_2 were calculated from the trough located cycle length data processed by the program ASTATA.BAS (Appendix C.1). The deviation of g_1 and g_2 from that expected for a normal distribution was assessed using the T test (for details of calculations see Appendix A.3).

For all six runs the observed third moments are positive and deviate significantly ($P< 0.05$) from that of a normal distribution, indicating that the distributions

are significantly skewed to the right (Table 4.3.3). In most cases (all four *L. acervorum*; one of two *L. tubero-interruptus*), the fourth moment departs significantly ($P< 0.05$) from the normal expectation. Since g_2 is observed to be positive, this statistic indicates significant leptokurtosis, that is, concentration of values close to the mean and in the tails of the distribution (in five out of six cases).

Table 4.3.3

Run	*n*	g_1	g_2	Sg_1	Sg_2
BIGRUN	*293*	*0.879**	*0.903**	*0.143*	*0.286*
MIDRUN	*270*	*1.080**	*3.393**	*0.149*	*0.298*
SMARUN	*263*	*1.236**	*2.661**	*0.151*	*0.302*
LITRUN	*277*	*0.859**	*1.367**	*0.147*	*0.294*
DIFRUN	*265*	*1.060**	*1.492**	*0.151*	*0.301*
TUBRUN	*218*	*0.545**	*0.102*	*0.166*	*0.332*

Analysis of third and fourth moments in activity cycle length distributions. Cycle lengths were measured from window X0. N: number of cycles observed; g_1*: third moment;* g_2*: fourth moment;* Sg_1*: standard error of third moment;* Sg_2 *standard error of fourth moment. For calculations of* g_1*,* g_2*,* Sg_1 *and* Sg_2 *see Appendix A.3.*

Hence analysis of third and fourth moments indicates that cycle length is not normally distributed; as suggested by Figure 4.7, it is strongly right skewed and leptokurtic. This result is relevant with respect to the use of autocorrelation and fourier analysis to investigate this phenomenon (see Section 4.5.1), and supports the prediction of Tofts (1990a) concerning cycle length distribution (see Section 4.4.3).

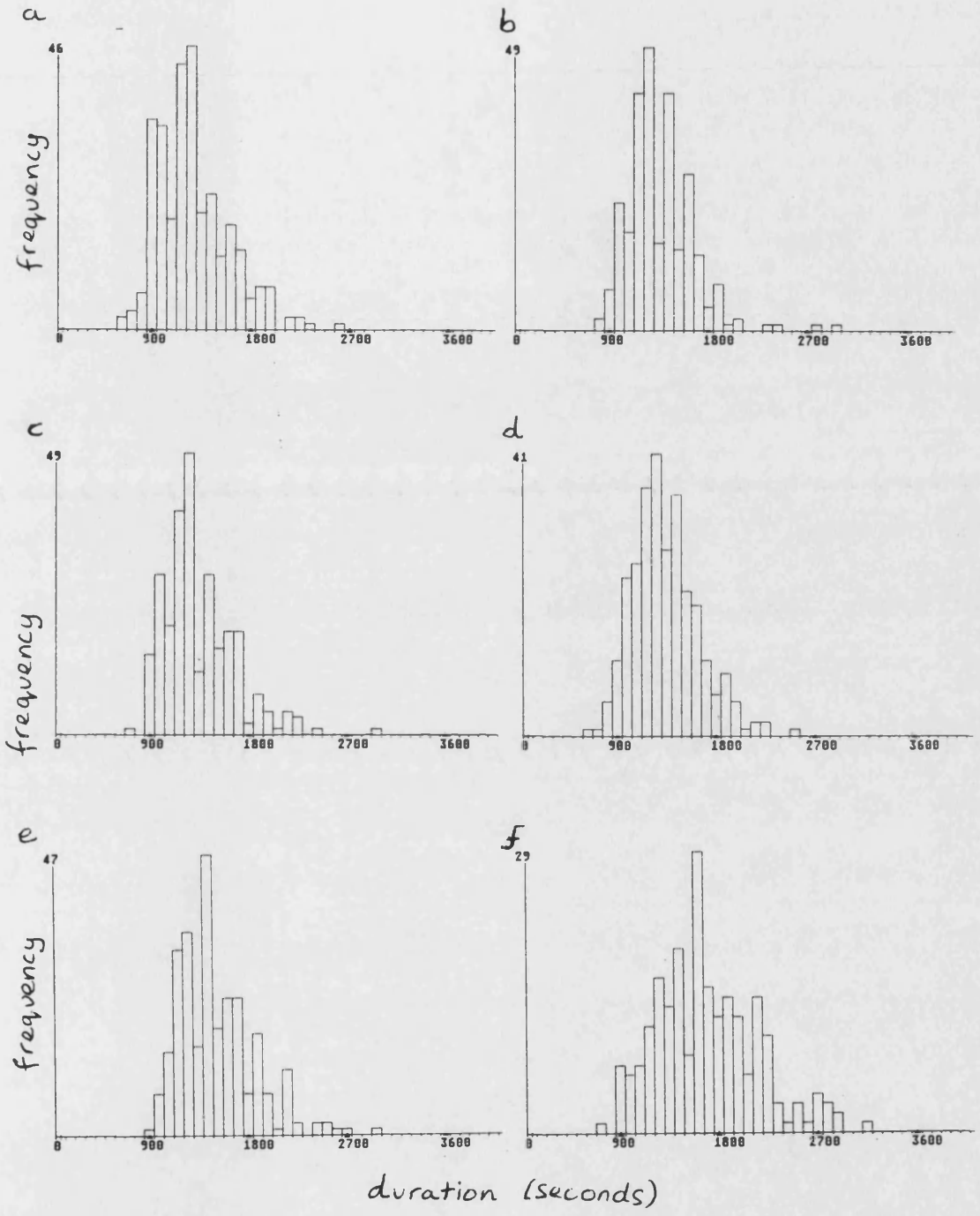

Figure 4.7: Activity cycle length distribution for completed runs *L. acervorum*:a, BIGRUN; b, MIDRUN; c, SMARUN; d, LITRUN. *L. tubero-interruptus*: e, DIFRUN; f, TUBRUN. Cycle length measures from window X0.

Log Survivorship Analysis

The distributions of cycle lengths (as estimated by the trough location procedure) for the six runs are represented as log survivorship curves in Figures 4.8. Two features are clear from inspections of these plots. Firstly, there is a distinct near horizontal "shoulder" to each curve, indicating that very few cycles are shorter than a certain fixed period. Once this duration is exceeded (see arrows on Figures 4.8), survivorship decays approximately in a straight line, and hence exhibits properties of exponential decay in the raw data distribution. The suggestion of exponential decay (or for discrete systems, a negative binomial distribution) to the cycle length data lends support to the model of Tofts (1990a), which predicts that cycle length will be distributed geometrically; the geometric distribution being a special case of the family of negative binomials. Further, the horizontal shoulder in Figures 4.8 supports the assumption Tofts (1990a) that the geometric distribution commences after a fixed lag period. This aspect is analysed further in Section 4.4.3.

4.3.7 Daily Activity Level

Although I have argued that any interpretation of cycle shape from rates of change of pixel mismatch should be regarded with extreme caution, consideration of changes in amplitude over the longer term may give insight into long term trends in the data. The mean activity level for all days increases with colony size (number of adults) for a given species (Figure 4.9), conforming to the expectation that higher numbers of pixel changes occur in larger colonies. These differences are often significant (Student's T test; comparing standard error of means for each run, $P < 0.05$ for all *L. acervorum* nests, except MIDRUN and LITRUN).

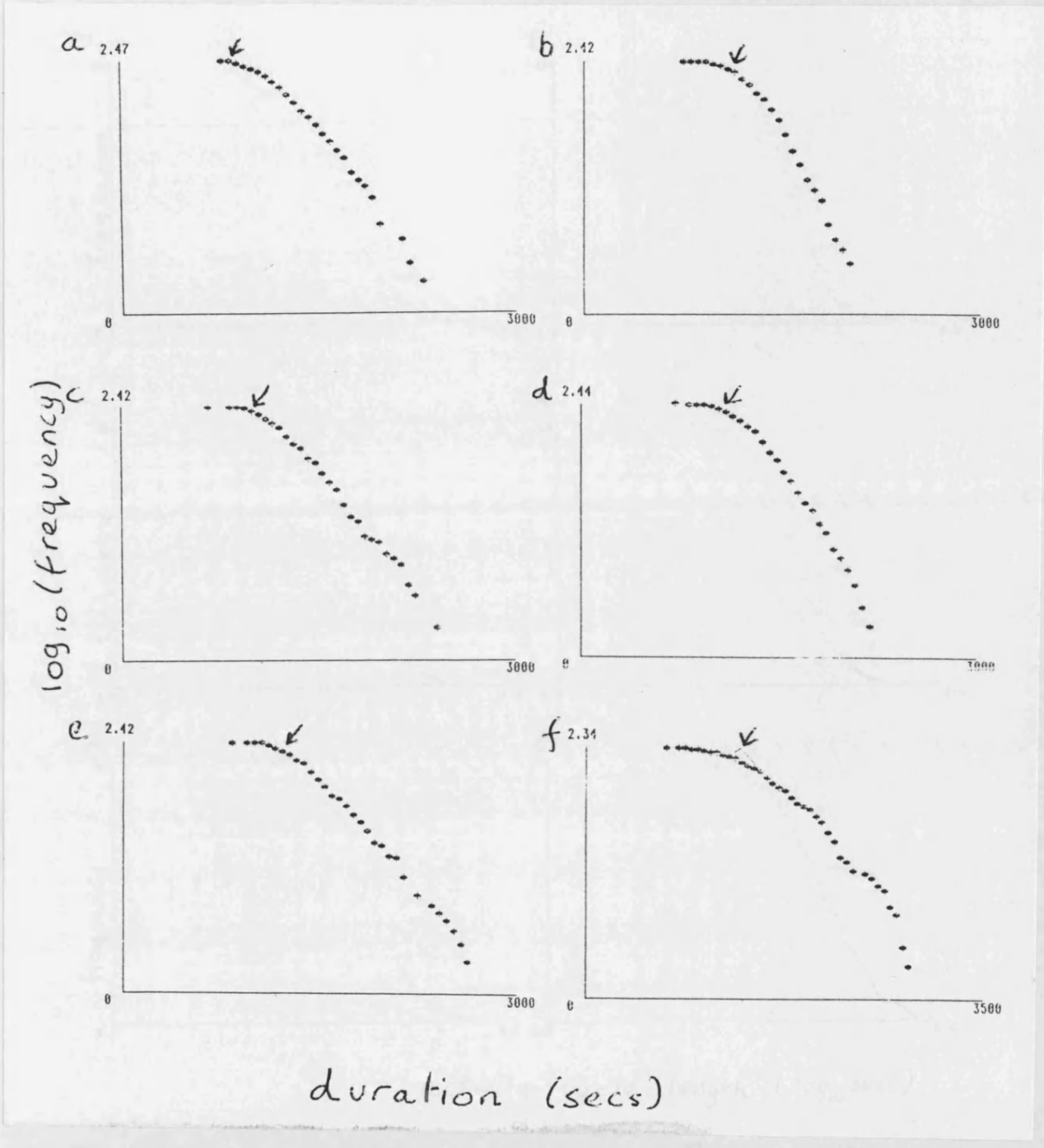

Figure 4.8: Log survivorship functions of activity cycle length. Cycle length (in seconds) measured from window X0. Each point shows the number of cycles that are longer than the cycle length shown on the horizontal axis. Arrows mark the point at which the horizontal shoulder intersects the straight line decay. *L. acervorum*: a, BIGRUN; b, MIDRUN; c, SMARUN; d, LITRUN. *L. tubero-interruptus*: e, DIFRUN; f, TUBRUN.

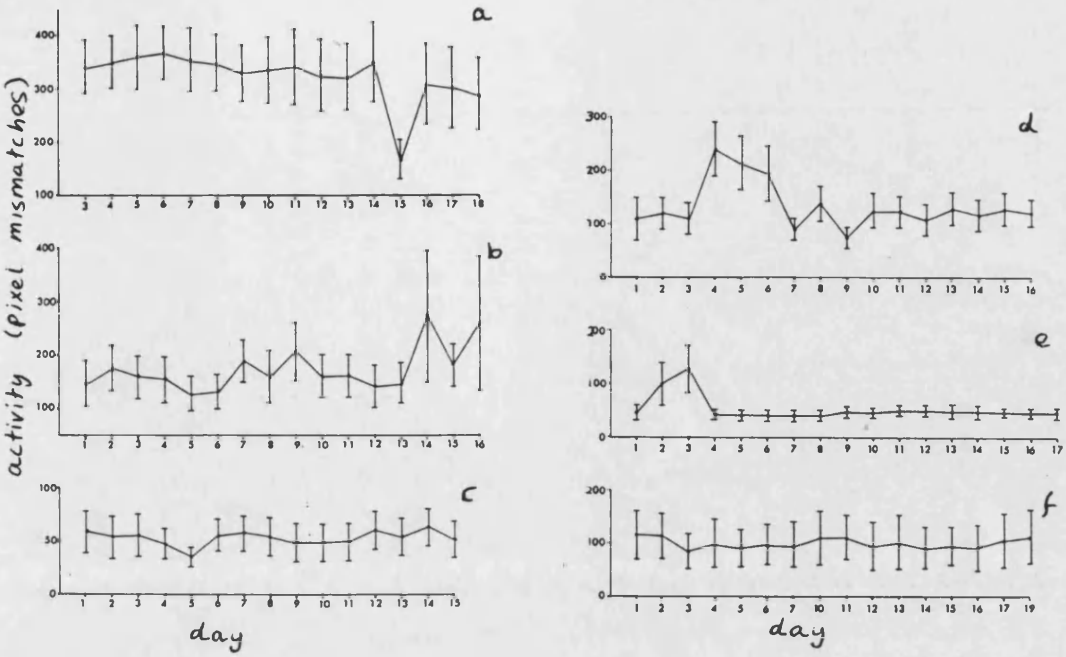

Figure 4.9: Daily activity level for each run. The mean and standard deviation of activity level (number of pixel mismatches) in window X0 are plotted for each day. a, BIGRUN; b, MIDRUN; c, SMARUN; d, LITRUN; e, DIFRUN; f, TUBRUN.

Relatively lower counts of pixel mismatches in *L. tubero-interruptus* may reflect the smaller size of these ants, or a lower activity level compared to *L. acervorum* (A.B. Sendova Franks, T. Stickland, pers. comm.).

Although there appears to be a slight trend downwards (Figure 4.9 a) or upwards (Figure 4.9 b) in some cases, there was no significant difference between the mean activity level in the first half of any run and that in the second half (Students T test; comparing standard error of the means for the first 8/9 days to those of the last 7/8 days, $P > 0.05$).

In small windows in particular, much of the activity may not be measured since ants may move straight through the window in less than the between frame interval (1 minute). Hence the number of pixel changes in Window 8 alone (en-

trance) is unlikely to relate to activity in that window, since most ants especially on entering the nest, run straight through this area (myself; N.R. Franks, pers. comm.). For this reason (and those explained in Section 3.1), comparison of pixel mismatches between different windows or for windows in different runs, or attempted measurment of "cycle length" within windows, is not particularly meaningful. We can however compare average number of pixel mismatches for given windows between days, interpreting the mean number of pixel changes merely as some indication of relative levels of activity. This is acceptable assuming that the nature of activity does not change particularly with time. In other words, provided that ants behave in the same way on different occassions within a given area of the nest (or petri dish), changes in pixel mismatch levels will relate broadly to changes in activity levels in that area. Mean levels of pixel changes for different windows for sample runs are presented in Appendix D.6. These analyses are again too numerous to present in their entirety. As for overall activity level, there appears to be no significant difference in activity levels in the brood pile, window X1 or outside the nest, between the two halves of each run (Students T test; $P > 0.05$).

4.4 Tests of Models

4.4.1 Simulation

Goss and Deneubourg (1988) predict: "when only a small number of individuals are present, the model generates synchronized but non-periodic activity". This "small number" is not quantified, but appears to imply "below 30" (S. Goss, pers comm.). Hence this prediction may be tested by ascertaining the existence or otherwise of cycles in a small colony (SMARUN, initially containing 12 ants;

see Figure 4.10, Appendix D.1). For further analysis of cycles within small groups of ants, see Chapter 7).

During the course of the SMARUN experiment, the number of adults increased from 12 to 23 as a result of eclosion of pupae. However, on the basis of Goss's (pers. comm.) comments, this still represents a "small colony".

Comparison between all data presentations discussed in Section 4.3 suggest no qualitative or quatitative differences in the patterns of activity in SMARUN and other larger nests of the species. The only notable difference is that mean daily activity is significantly lower (Figures 4.9) in the small nest, as would be expected since fewer ants contribute to daily activity. However, the temporal pattern of activity does not appear to differ. The raw data activity time series for the whole nest (sample shown in Figure 4.10, see also Appendix D.1) exhibits clear fluctuations between relatively low and high levels of activity, and is similar in form to time series from other nests. Analysis of turning points (Table B.0.3) indicates that all daily time series are not random, in that all turning point scores are significantly lower ($P< 0.05$) than the random expectation.

Autocorrelation results (for sample correlograms see Appendix D.3) and first return maps (Figure 4.6 and Appendix D.4) do not appear to differ qualitatively from those generated by larger nests, and again indicate cyclicity in the time series. The mean cycle length (based on cycle length estimates from the trough location procedure) does not differ significantly ($P> 0.05$) from that of other *L. acervorum* colonies (see Table 4.3.2)

The distribution of activity cycle lengths (Figure 4.7 c) again appears similar to those for larger colonies. Cycle length in SMARUN is not distributed nor-

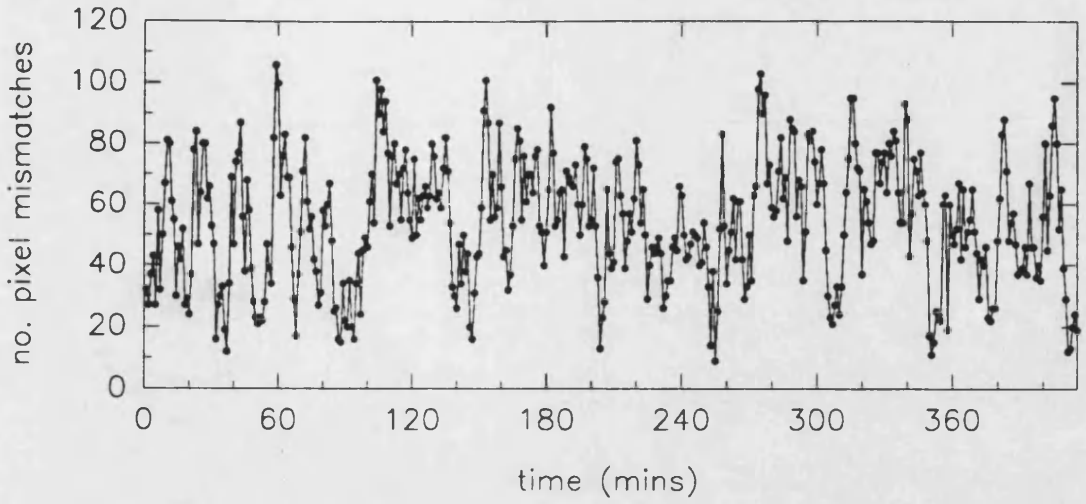

Figure 4.10: Activity time series in a small nest of *L. acervorum* (window X0). The time series represents day 02 of the run SMARUN; this colony initially contained twelve workers.

mally, exhibiting positive third and fourth moments that both depart significantly ($P < 0.05$) from the normal expectation (Table 4.3.3). Hence, cycle length in SMARUN exhibits a right skewed leptokurtic distribution, as found for the other *L. acervorum* cases. The log survivorship plot of cycle length consists of a near horizontal shoulder followed by a linear decay, in common with those of other colonies, suggesting that cycle length is distributed as an exponential decay after a fixed lag period. Hence, there appears to be no evidence in support of Goss and Deneubourg's (1988) prediction that small colonies will not exhibit cyclical activity.

4.4.2 Activity linked to Energy Level

Hemerik et al. (1990) predict that as brood to worker ratio increases, cycles should occur at higher frequencies, and will eventually break down at high brood to worker ratios. This critical ratio is not reported, since it will depend on the rates of calorific energy intake into and utilization within the nest, which have

not been measured. The data does not support this prediction; it is not possible to discern differences in cycle duration for colonies of different brood to worker ratio, however this ratio is quantified (see Tables 4.3.2 and 4.4.1).

Table 4.4.1

Run	*Total Brood / Worker(i)*	*S+M+L / Worker (ii)*	*mean cycle length*
BIGRUN	*1.57*	*0.86*	*1239*
MIDRUN	*1.75*	*1.54*	*1307*
LITRUN	*1.85*	*1.83*	*1305*
SMARUN	*4.67-1.09*	*2-0.87*	*1326*
DIFRUN	*1.17*	*0.62*	*1470*
TUBRUN	*0.88*	*0.72*	*1642*

Ratio of brood items to workers (2 estimates) in each run. (i) total brood to worker ratio, brood is the sum of eggs and small, medium and large larvae and pupae; (ii) brood is the sum of small, medium and large larvae only, hence this estimate includes only brood that require feeding.

Possibly a more rigorous test of this model is related to its assumptions rather than its predictions. The model assumes that foraging level is (linearly) proportional to the total number of ants active in the nest. Pearson Product Moment correlation coefficients were calculated between pixel mismatch levels for relevant windows (i.e., inside the nest versus outside the nest - see Figure 4.1 and Table 4.2.3) to test this assumption.

Table 4.4.2 a-f summarizes information obtained from correlating pixel mismatch levels between various windows. The number of pixel mismatches in window 5 and 6 (forage and water tubes) does not correlate appreciably with that in

windows within the nest (1, whole nest; 2, brood pile; X1 nest minus brood pile). Activity outside the nest correlates on roughly one third of occasions with that inside, although rarely with that on the brood pile. Activity directly at the nest entrance (X3) correlates with that inside the nest on approximately 60% of occasions, although again this correlation appears chiefly within the area away from the brood pile. These results do not support the assumption of Hemerik et al. (1990) that foraging activity is linearly proportional to overall colony activity level.

Table 4.4.2

(a)

	1	2	X1
X2	2	-1	4
X3	14	4	14
5+6	1	0	-1

(b)

	1	2	X1
X2	11	6	12
X3	9	3	10
5+6	-1	0	-3

(c)

	1	2	X1
X2	9	3	14
X3	14	4	16
5+6	1	4	1

(d)

	1	2	X1
X2	3	-1	9
X3	9	1	13
5+6	-1	-3	0

(e)

	1	2	X1
X2	3	1	3
X3	3	2	2
5+6	1	1	1

(f)

	1	2	X1
X2	6	5	14
X3	8	8	16
5+6	-1	-1	-2

Correlations between activity level in various windows. Scores indicate the number of significant correlations between pairs of windows (as marked). Runs a, BIGRUN(16); b, MIDRUN(16); c, LITRUN(16); d, SMARUN(15), e, DIFRUN(17); F, TUNBRUN(16). The maximum possible number of correlations is given in brackets beside the run name. Scores prefixed - denote negative correlations.

4.4.3 Algebraic Description

Tofts (1990a) predicts that cycle length should be (fairly) independent of colony size and distributed as a geometric distribution, commencing its decay after a fixed time period s. The former prediction is supported by the data (Table 4.3.2) indicating no significant difference between mean cycle length for *L. acervorum* colonies of differing size. The latter prediction was tested using the χ^2 goodness of fit test encoded in the program ASTATA.BAS (Appendix C.1). The expected distribution was calculated on the basis of the known characteristics of geometric distributions; in conjunction with estimates of base sleep time s, cycle length mean and variance from the data (see Appendix A.4).

The expected distribution was formed by calculating expected frequencies in each of ten equal intervals in the range 0 to $max1$ ($max1$ is the maximum observed cycle length minus s; see Figure 4.11 a). Similarly, the data is reformed into a frequency distribution consisting of 10 equal intervals ranging for 0 to $max1$. This requires that s is subtracted from each datum point (i.e., each measurment of cycle length). If the observed cycle length is less than the estimated s, a negative value will result. These negative values are counted as occuring in the first interval of the observed distribution.

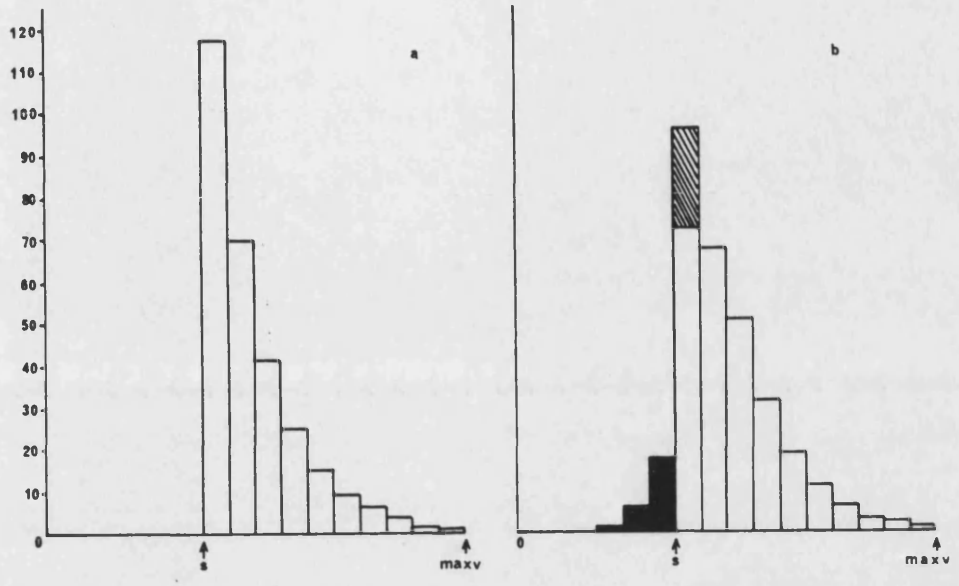

Figure 4.11: a, Expected geometric distribution for cycle length. The constant s is estimated from the data mean and variance (see text for details). The expected frequency in each interval is calculated from the pdf for the geometric distribution and the sample size. b, Fitting observed data to testable intervals of the geometric distribution. Observed values (black) that lie below s (estimated from the data and model) are included in the first testable interval (hatched). Counts in each interval are compared to the expected distribution by a χ^2 test. Observed and expected distributions shown: BIGRUN.

Since the goodness of fit is tested by χ^2 comparison of the observed and expected distributions, we require that all intervals have expected value greater than five (Sokal and Rohlf, 1981:709). This was assured by summing the expectations of the last three intervals (i.e., those with the longest cycle length) to produce a single cell. Hence, in those cases, goodness of fit was tested by comparing the observed number O_i of values in each interval (again, summing the last three intervals), to that generated by the expected distribution (E_i):

$$X^2 = \Sigma_{i=1}^{i=7} \frac{(O_i - E_i)}{E_i}$$

The value of χ^2 is compared to the critical value χ^2 (P=0.05,5df). The number of degrees of freedom is n-2 (n= no. of intervals) since the expected distribution has been generated utilizing parameters estimated from the observed data (Sokal and Rohlf, 1981:715).

Our estimates of p (probability of waking) and s result from substitutions in the equations for mean and variance of a geometric distribution, and the value of variance (v_{data}) is estimated from the data. v_{data} itself is bounded by an error range ($v_{data} +/- t \times standard\ error\ on\ v_{data}$). Hence, we can construct lower and upper limits to our estimates for p and s, by substituting appropriately into the equations for lower and upper variance bounds (For details see Appendix A.4). The combination of p and s values from three estimates of each yields nine possible distributions; a maximum likelihood distribution and eight further distributions at the bounds of the error range. These distributions were also calculated and χ^2 test performed on each by the program ASTATA.BAS, yielding nine goodness of fit values for each run. The calculation of these values allows us to locate more precisely which values for the model's parameters produce a good or a poor fit to the data.

As on first inspection, the inclusion of negative values in the first interval may appear to increase the likelihood of concluding that the data fits the expected distribution (see Figure 4.11 b), it is neccessary to justify this procedure. It would be extremely difficult (as well as somewhat questionable) to ignore any data values (for instance, if they were considered to result from human error in measurement). This is because it is not possible to identify "erroneous" values until s has been calculated. The value of s depends on the mean and variance of the distribution, to which all values contribute. Hence if "erroneous" values were ignored, s would have to be recalculated, on the basis of the revised data set. A revised value of s could yield further "erroneous" values; such a procedure may therefore have to be repeated indefinately. Clearly a cut off point to its repetition would require further subjective decisions. Secondly, if data values which are too small to fall into one of the testable intervals must be included, it is reasonable to pool them in the nearest testable interval.

The results of the goodness of fit tests are presented in Table 4.4.3. For *L. acervorum* in two out of four cases, the observed distributions (see Figure 4.7) depart significantly ($P< 0.05$) from that expected, based on the maximum likelihood estimates of p and s. For *L. tubero-interruptus*, both distributions depart significantly from the maximum likelihood estimator. However, in all runs apart from TUBRUN (*L. tubero-interruptus*), at least one distribution can be found using allowable estimates of p and s which fits the data ($P> 0.05$). When the relative values of s and p that provide well fitting distributions are plotted (Appendix A.4), areas of best fit are located to high values of s and the region of p for the central to the upper limit (in all cases except TUBRUN). Hence, a better approximation to the data is obtained using a longer sleep time than that directly estimated from the data using the model.

The duration of the lag phase s can also be estimated from log survivorship curves of cycle length. We can interpret the horizontal shoulder in these plots (Figures 4.8) as representing the region (in terms of cycle length) that is shorter than s. Hence, the point of intersection between the shoulder and the line of more rapid decay should represent the position of s. Using this technique (fitting lines by inspection), I estimated s from the log survivorship curves (Figure 4.8). The estimates of s from the log survivorship and probabilistic model compare favourably (Table 4.4.4), in that they do not differ by more than one minute (the error from measurement in the original time series being +/- 1 minute).

The observed values of g_1 and g_2 (Table 4.3.3) are consistent with the interpretation that cycle length is geometrically distributed, since they suggest right skewness, leptokurtosis and departure from normality. However, the direct test of the WSCCS model presented in this section clearly suggests that it would be unwise to accept the model without reservations, since not all data sets conform to the most likely distribution. It would also seem unreasonable to reject the model entirely at this stage, since other estimates of parameters yield a good fit to the data, independent estimates of the lag phase agree within the bounds of measurement error, and statistics of third and fourth moment support the interpretation of a geometric distribution.

Table 4.4.3

Run	*N cycles*	*Mean length*	*BST (s)*	χ^2 *MLE*	χ^2 *best*	*No.* χ^2 < 11.07	*No. short cycles*
BIGRUN†	*293*	*1239*	*912*	*11.03**	*3.95**	*5*	*24*
MIDRUN†	*270*	*1307*	*1043*	*10.57**	*5.17**	*4*	*19*
LITRUN†	*277*	*1326*	*1044*	*17.33*	*4.82**	*3*	*17*
SMARUN†	*263*	*1305*	*982*	*11.8*	*6.96**	*3*	*14*
DIFRUN•	*265*	*1470*	*1129*	*12.0*	*9.47**	*2*	*22*
TUBRUN•	*218*	*1648*	*1185*	*24.94*	*11.22*	*0*	*24*

*Summary of goodness of fit tests of WSCCS model. Cycle length measures in seconds from window X0. Base sleep time (s) estimated from the data using the model (maximum likelihood estimate). Nine test distributions were calculated from each data set; the value of χ^2 for the maximum likelihood distribution (MLE) and the best fit distribution (best) are given in each case. * indicates no significant departure between expected and observed distributions. The number of permissable test distributions (out of nine) that fit the data by this criterion are also given. The number of cycles that are shorter than the base sleep time are also shown.*

Table 4.4.4

Run	Log Estimate s_l	Model estimate s_g	$s_l - s_g$
BIGRUN	935	912	23
MIDRUN	1066	1043	23
SMARUN	959	982	-23
LITRUN	1000	1044	-44
DIFRUN	1131	1129	2
TUBRUN	1222	1185	37

Comparison of estimates of base sleep time. Estimates of s are given in seconds. Log survivorship estimate of s (s_l) was taken as the point of intersection of straight lines differing in slope; from log survivorship curves. The model estimate of s (s_g) was calculated from the cycle length data, using known parameters of the geometric distribution. Cycle length measured from window X0.

4.5 Discussion

4.5.1 Implications of results for the models

Temporal pattern of activity within nests

The results presented in Section 4.3 are in agreement with the findings of Franks and Bryant (1987), Franks et al. (1990a) and Cole (1991a), that activity level within leptothoracine nests varies nonrandomly, and cycles over a 'period' of roughly 20 minutes. Activity level (measured as number of pixel mismatches between 1 minute intervals) follows a nonrandom course in time (Turning points: Tables 4.3.1 and Appendix B.0.3). Cyclicity is suggested by the form of activity timeseries (Figures 4.3 and Figure 4.10) and autocorrelation of these time series, revealing significant correlations at time lags of 15-25 mins (Figure 4.2 c and Appendix D.3). However, it is also clear from inspection of time series plots, that the period from one activity peak (or trough) to the next varies considerably (as suggested by Franks et al., (1990), Cole (1991a), from comparison of correlograms). Cycle length is not distributed normally, being right skewed and leptokurtic (Table 4.3.3). The form of log survivorship curves also suggests that cycle length is distributed as some form of negative binomial, rather than normally. The distributions can be fitted to a geometric distribution with a fixed lag component s, and estimates of s from model fitting and log survivorship curves tend to agree within the bounds of measurement error. Hence the variance of the measurement (trough-trough interval) is large compared to that of a normally distributed variable.

This result presents us with the difficulty of providing a semantically correct de-

scription of activity patterns within nests. I consider this problem in an attempt to avoid the use of terms the meaning of which is too strong to be supported by the data. Although described as "periodic" (Cole, 1991a), the pattern of activity observed is not strictly so, since the period is neither fixed nor varying normally within a small range about its mean. Similarly, its description as "rhythmical" (Franks and Bryant, 1987; Hemerik et al., 1990; Goss and Deneubourg, 1988; Cole, 1991a) may not strictly be appropriate. The activity pattern may be described as "cyclical" (Tofts et al., 1992), in the sense that it results from cycling between two states: colony activity and inactivity. On the basis of fit to Tofts'(1990a) model, the interval between the recurrence of a given state appears to be determined probabilistically after a fixed period s has elapsed.

On initial inspection, the activity time series suggest that the states 'colony activity' and 'colony inactivity' are relative terms only: the number of pixel changes does not decay to zero, and the maxima are of the order of 3 times that of the minima. As suggested in Chapter 3, the relationship between activity level and number of pixel changes is not linear; high levels of activity are likely to be underestimated by pixel mismatch counts. At the magnifications used in these experiments, a single ant covers approximately six pixel blocks (4×4 squares). Hence, a single ant moving to a new position may record up to 12 pixel mismatches, so 100 pixel mismatches may represent as few as nine active ants. However, as more ants become active and begin to overlap in position, underestimation of activity increases. From direct comparison of observations and pixel mismatch levels, a pixel mismatch level of 300 may represent 60 active ants. Hence it is likely that colony activity level varies considerably more than the threefold change suggested by digitized measures. However, nonzero pixel change counts during troughs suggest that low levels of activity occur in the 'inactive' phase, since the filtering techniques described in Chapter 3 reduce false positives

to very small levels.

This suggests that the description of colony activity as "bursts" or "pulses" of activity (Franks et al., 1990a; Hemerik et al., 1990) between regions of complete inactivity is too simplistic. Also it suggests that the simplifying assumption that individuals are identical with respect to their waking behaviour in the models of Goss and Deneubourg (1988) and Tofts (1990a) may be incorrect. This feature is investigated for individuals in Chapter 5.

Inspection of time series suggests highly variable amplitudes to activity cycles, and in some cases (for example Figure 4.3 a) beating between low and high amplitude waves. The phenomenon of beating suggests that the overall pattern of activity is composed of two or more underlying cycles of differing frequency, which tend to cancel or compliment as they change relative phase to each other.

There is some indication from the time series and return maps of individual regions within the nest that the ants on the brood pile and near the entrance cycle at different rates (this possibility is investigated in Chapter 5). Low correlation scores between activity in different parts of the petri dish indicate that activity throughout the nest and exterior may not be as synchronized as previously suspected (Franks and Bryant, 1987; Cole, 1991a). These correlation patterns again suggest that several groups of ants are somewhat decoupled from each other in terms of activity synchronization, so leading to a complex pattern of activity cycling overall.

Hence, we can describe the activity pattern within nests as cycling between low levels and high levels of activity approximately every twenty minutes (for *L. acervorum*). Cycle duration and amplitude are highly variable, and there is

a suggestion that the overall pattern of activity may result from interactions between two or more groups of ants, which tend to differ in characteristic activity patterns.

Simulation Model

Contrary to this model's prediction, cycles of activity that were indistiguishable qualitatively or quantitatively from those in larger colonies were found to occur in a nest of 12 to 23 workers. As explained below, it is not clear that the mechanism underlying the model will lead to the outcome of non-cyclicity in small nests, rather this may have resulted from the model's implementation. It is hard to judge whether any of the data I have presented contradict the model, since its predictions are described using terminology which is imprecise. Since the basic mechanism of autosynchronization underlies the model of Tofts (1990a), it is perhaps better to consider its applicability with reference to that model instead.

Energy Model

The results presented do not suggest any trend in cycle length with brood to worker ratio, and thus do not support the prediction of Hemerik et al. (1990) that cycle duration decreases with increased brood to worker ratio. Also, at no point did cycles appear to break down, as suggested for high brood to worker ratios. However, I cannot judge whether any of the ratios investigated were large enough compared to theoretical ratios that might yield this phenomenon. In Chapter 6 I investigate the effect of increasing energy demands on colony activity patterns by starvation of the colony, since continued deprivation should

allow such theoretical thresholds to be reached.

Algebraic Description

The distributions of cycle lengths in *L. acervorum* are broadly in agreement with the prediction of Tofts (1990a). In 5 cases, each based on over 260 observed cycles, the distribution did not differ significantly from some predicted by the model. However, in one run for *L. tubero-interruptus*, cycle length was distributed in a way that differed ($P<0.05$) from all nine predicted distributions.

Frequency distributions and log survivorship indicate that in all 6 cases the distribution of cycle length clearly contains a geometric (exponential decay) element, however the minimum cycle length is not the mode: there appears to be some "early waking" of the system. To some extent such "early waking" may be the result of measurement errors, but the model's rejection in 2/4 and 2/2 cases (maximum likelihood distribution) for *L. acervorum* and *L. tubero-interruptus* respectively calls the success of this model into question.

On average, 7.5% of all cycles fell below the lag length s. However, many of these short cycles are within two minutes of s (see Table B.0.6), and may be accounted for by errors in measurement. The remaining short cycles (roughly 2% of all cycles) may indicate rejection of the WSCCS model, but alternatively may have resulted from disturbance to the colony (for example, vibrational disturbance when other researchers entered the room).

The general shape of the cycle distributions suggests that probabilistic elements are important in determining the onset of activity bouts. This distribution is con-

sistent with the model's general mechanism, that individuals with probabilistic waking after a lag time interact resulting in autocatalytically generated activity cycles. Further, the close agreement between two independent estimates of s is strong support for the notion that s is a relevant parameter in this system.

Lack of precise fit to the model in some instances and nonzero measures of activity during 'inactive' phases suggests that although the general principle of the mechanism may be sound, certain of the model's assumptions may be too simplistic. Firstly, autocatalysis may not be strong: individuals may frequently wake independently. Secondly, the assumption that individuals possess a fixed minimum rest period before they are receptive to 'waking' may be incorrect. Also, more complex behaviour at the colony level may result from a more complex combination of simple units: individuals may not behave identically with respect to their activity pattern. Examination of these possible sources of mismatch between the model and observed activity patterns requires study of individuals, and is described in Chapter 5. Lastly, the assumption that the waking signal is fast compared to cycle duration may be erroneous. I return to this question in Chapter 7.

4.5.2 Data Interpretation

Pixel mismatches in small windows.

From Table 4.4.2, we can conclude that the pixel mismatch level in the window containing the food and water tube does not correlate to that within the nest. However, only cautious interpretation of pixel mismatch level as foraging level can be made. Firstly, we cannot assume that all activity in that window relates

to foraging. In such laboratory nests, I have frequently observed groups of ants to gather at water tubes in particular, and not exhibit apparent foraging (or drinking) activity. Pixel mismatch level in window 5 may also reflect transient movement of "patrolling" ants in this area (Gordon, 1987) Foraging activity itself may be underestimated, since ants that forage within the cotton wool plug of the tube do not always produce an image on the camera or digitizer. Due to these reasons, and the small size of this window, pixel mismatch levels in this window were frequently very low (Appendix D.2). In addition, other measurements that may relate to foraging activity were compared to activity within the nest (Table 4.4.2). Significant correlation occurred between windows X3 and X2 quite frequently, suggesting that workers within the nest not involved in brood care are connected with activity directly outside the nest. This activity may often represent only transient movement at the entrance, rather than activity in the foraging arena in general (Herbers, 1983). Further, it is in the area of the brood pile that activity is most highly synchronized and cyclical. Therefore it seems reasonable to conclude that foraging level is not generally proportional to activity in the nest; since most changes in within nest activity levels result from nurses, and their activity is not correlated to that outside the nest. I examine this isssue again in Chapter 5 and 6.

Autocorrelation and Fourier Transform

The duration of activity cycles is one of the parameters more amenable to measurement by automated image analysis techniques. However, the suspected nature of cycle length distribution itself makes the use of standard techniques inappropriate.

The maximum value of correlation coefficient will indicate the modal lag at which correlation occurs. Hence, for rhythmic data with normally distributed period, this maximum coefficient will indicate the mean cycle duration. However, length of activity cycles in ants does not appear to be normally distributed. Goss and Deneubourg (1988) and Hemerik et al. (1990) do not describe the expected distribution, but it is unlikely to be normal, certainly in the case of Goss and Deneubourg's model (1988) (C. Tofts, pers. comm.). Tofts (1990a) predicts specifically a geometric distribution; here, clearly the mode corresponds to the minimum period and is not equal to the mean. Hence autocorrelation of activity time series will yield estimates of minimum cycle length, rather than mean. Franks and Bryant (1987), Franks et al. (1990a) and Cole (1991a) utilize autocorrelation to estimate activity cycle length in ants. They conclude that cycle length is highly variable, as would be expected on the basis of small data sets with a variable minimum cycle duration. Comparison of autocorrelation results (lags at which significant correlations occur) and mean estimation for my own data confirm this argument: significant lags (in minutes) are generally shorter than the estimated mean cycle duration.

Fourier decomposition of the time series is designed to decompose the series into one or more sinusoidal elements, and hence detects strong sinusoidal wave components (Kendall, 1976:95; Chatfield, 1984:127). The equations of Hemerik et al. (1990) do not yield a sinusoidal wave form. Goss and Deneubourg (1988) do not make explicit the nature of their resulting wave, but by visual inspection of the simulation output, and considering the nonlinearities encoded therein (positive feedback in the "waking" signal), I would not expect it to be sinusoidal. Tofts (1990a) also results in a time series that is clearly not composed of sine waves. Interpretation of a Fourier decomposition which yields the relative strengths of sine waves of different frequency that compose a time series would therefore be

inappropriate (Enright, 1981). Cole (1991a) applies this method of analysis to activity patterns in colonies of *L. allardycei* . From the data presented in his paper, there appears to be little similarity between decompositions for different nests under similar conditions; an observation which is as consistent with the notion that this analysis technique is inappropriate as it is with the conclusion that underlying cycle form differs between experiments (see also Bennet-Clark, 1990; Enright 1990; concerning problems of Fourier interpretation).

Measurement of Cycle Length

In order to arrive empirically at a distribution of cycle length, it was thus neccessary to develop an alternative method to measure cycle length. This method itself is open to question, in that it relies on the (possibly subjective) interpretation of the time series by an observer: local minima are located by eye, after initial processing of the data to make such minima clearer. There seems to be no obvious solution to this problem. A generalized automated procedure for locating minima is not available; in a sense the use of automated procedures is also subjective, in that firstly, the observer must decide how "local" a minimum should be. In an attempt to quantify the extent of subjectivity in trough location, I carried out blind tests to determine the repeatability of minima choice (and hence, cycle length determination). These results are presented in Appendix A.5. In summary, these tests did not reveal a significant subjective component to the measurment of cycle length by location of local minima, since trends in cycle duration were maintained when files were presented to the observer (myself), unlabelled and in random order. Hence, although a measurment technique which does not involve human choice would be preferable, the neccessary intervention here does not generally appear to invalidate the results.

4.5.3 Some Aspects of Model Interpretation

Definition of Terms

Goss and Deneubourg (1988) provide the first possible explanation for activity cycles involving mechanism. The mechanism of autocatalysis (active ants stimulate inactive ants, leading to positive feedback) is quite plausible. Also, the model suggests that only simple behaviour on the part of individuals is neccessary to generate cyclical activity patterns at the colony level, and it does not require us to assume that individuals have knowledge of colony level parameters in order to interact in this fashion. However, the model's validity is difficult to assess, since the form of expected cycles is not presented. Goss and Deneubourg do suggest that "synchronized but non-periodic" activity will occur in "small" colonies, but the precise meaning of these words is not clear. Conversely, they do not make clear what "periodic" entails with respect to larger colonies.

Model Implementation

The mechanism underlying Goss and Deneubourg's model is implemented as a computer simulation. Since the mechanism involves probabalistic elements, the results of simulations for a given set of starting parameters (e.g., colony size, wake and sleep probabilities) will vary. In essence, a single simulation represents a single "experiment", that is, an individual outcome sampled from a population of possible outcomes. Goss and Deneubourg present only one such sample in their paper, and do not indicate the variation in, say, cycle length when a larger number of such samples are pooled. There is also no formal proof that the mechanism will

result in "periodic" activity for colonies that are not "small". Thus comparison of any measurements from the data to any measurments produced by simulation of the model is not possible.

With reference to the prediction that small colonies do not exhibit periodic activity, the precise form of implementation has to be considered. Ants were "randomly paired" (Goss and Deneubourg 1988) by allowing them to perform random walks across the computer screen until they met another such individual (S. Goss, pers. comm.). Thus the computer screen acted as a fixed nest space. Consequently smaller colonies occupied the same area as large colonies, and thus area per ant was greater for ants in small colonies. Here, lack of periodicity in small colonies may be the result of decreased probability of encountering another ant, rather than the (implied) decreased probability of any individual waking at a particular instant. In reality, small leptothoracine colonies tend to occupy proportionally smaller areas; the individuals are not particularly over-dispersed compared to individuals in colonies of larger size (myself, N.R. Franks, A.B. Sendova-Franks, pers. comm.).

Parsimony and Predictive Power

Hemerik et al. (1990) make explicit the mechanism underlying their model, and attempt to arrive at testable predictions after mathematical analysis of the model's behaviour. However, its predictive power is still limited, in the sense that the particular predictions it makes are not easily amenable to experimental test. Firstly, parameters important to the predictions are hard to quantify, for instance the critical brood to worker ratio or level of starvation at which cycles break down. The consequence of this is that further data must be gathered

(concerning rates of increase and decay in the colony calorific energy store) to quantify these thresholds. Further difficulties in the experimental test of this model (in that parameter changes for starved colonies are difficult to interpret from the data) are discussed in Chapter 6.

Hemerik et al.'s model is formulated from 7 constants in 2 coupled nonlinear differential equations. For particular values of the constants, cyclic activity patterns emerge. However, the biological meaning of these constants is not clear; hence their value cannot be determined experimentally. This complexity also results in a confusing array of possibilities for alteration if a mismatch is found between observed behaviour and that predicted by the initial model. Thus, due to its initial complexity, the model's refutation does not suggest immediate avenues for improvement.

As discussed in Section 4.1, implicit in the formulation of the coupled differential equations is the assumption that the number of active foragers is proportional to the level of activity within the nest. Correlation results presented in Table 4.4.2 do not support this hypothesis. Also implicit in the mechanism is that individuals have "global knowledge" of energy levels: the behaviour of an ant is determined by the energy level of the colony, not of the individual itself. Hence each individual must sample sufficient information on the whole colony's nutritional status. In species that exhibit a division of labour (e.g., between castes that forage and those that tend brood), this information will be indirect, at best.

Although individuals may retain an estimate of such global parameters as a result of interactions with other individuals, such sampling would require time to achieve a reasonable approximation, by which point it may be out of date. Hence the fundamental premise of the model, that a relatively long term vari-

able (colony food level) determines a short term variable (colony activity level) requires consideration.

Such linkage of short and long term variables would be interesting since it might suggest that colony activity level was part of a foraging mechanism, enabling colony response to fluctuations in resource level. However, it is not clear that such a linkage would lead to efficient response, or be selectively advantageous. This foraging strategy differs fundamentally from standard forms of satisficing strategy *eat until you will not be hungry for a given period* or an optimizing strategy *eat until the costs of continued eating outweigh the benefits* (Charnov, 1976; Krebs and Kacelnik, 1991). Rather Hemerik et al.'s ants utilize the strategy *do not eat until hungry.* Since individuals require some time to assess when the colony is hungry (sample sufficient information) and alleviate hunger (forage successfully), such a strategy may not be beneficial, since survival of brood may be impaired.

Although it is reasonable at some point to attempt to account for the evolution of a phenomenon (*vis a vis* selective advantage) it is not neccessarily appropriate to attempt to build such adaptive reasoning into models of the mechanism underlying the proximate cause of the phenomenon. Increasing model complexity by incorporating both ultimate and proximate causes may reduce the possibility of refutation of the model and of either cause. Inclusion of ultimate causes at this stage may be at the expense of simplicity in the underlying proximate mechanism, thereby leading to the acceptance of an ultimate cause for which there is no evidence whilst assuming more complexity on the part of individuals than is neccessary to explain the phenomenon. In analogy with "hitch-hiking" (Maynard Smith, 1978); where alleles of no adaptive significance increase in the population by virtue of their physical association with alleles that are actively selected, one is lead to the conclusion that here, adaptive reasoning has hitch-hiked in associa-

tion with mechanistic explanations of the biological phenomenon. I will consider possible adaptive advantages of synchronized and or cyclical activity patterns in Chapter 8.

The WSCCS model of Tofts (1990a) makes no attempt to incorporate ultimate causes into mechanism. Indeed, one might argue that it makes no attempt to incorporate biological feasibility into mechanism (this is recognized by the author; Tofts, 1990a; Tofts et al., 1992). In order to retain a simple, analysable system, Tofts makes the assumption that, *an active ant will wake all other wakeful ants.*

Hence the ants of Tofts can stimulate arbitrary many ants at an arbitrary distance. The social spider *Anelosimus eximus* may however satisfy this condition naturally; spiders synchronize their activity within prey retrieval bouts, probably through response to vibrations on the web (Krafft and Pasquet , 1991). Here, the assumption is made in order to allow tractable analysis of the model, such that testable predictions can be made enabling its refutation. In real terms, the assumption can be phrased as, *the time scale of any possible waking mechanism is much shorter than the length of the cycle* (Tofts et al., 1992).

In Chapter 7 I attempt to analyse this requirement experimentally. The highly simplistic model of individual ants in Tofts (1990a) allows the interaction of such individuals to be analysed without incorporating additional assumptions about their behaviour, such as the random walk assumptions made by Goss and Deneubourg (1988) that may lead to problems in encoding the mechanism. Hence although the assumptions may appear unrealistic, they yield a simple, well defined model of individuals, and allow complete analysis of their interactions. Since the model makes testable predictions, it is refutable. As very few factors are initially involved, their contribution to the behaviour of the model can be examined

to indicate possible sources of mismatch between empirical data and model behaviour. If experimental data do not indicate rejection of the model, it remains as the most parsimonious explanation of the phenomenon, in that it assumes minimal behavioural complexity of individuals, or complexity of interactions.

Chapter 5

Measurement of Individuals

5.1 Introduction

The WSCCS model (Tofts, 1990a) presented in Chapter 4 includes a number of simplifying assumptions concerning the behaviour of individuals. In Chapter 4, I conclude that there is a lack of precise quantitative fit between observed data and the model's predictions for colony activity. In order to test the assumptions concerning the behaviour of individuals, measurement of individual parameters is required. Hence the image analysis techniques used in Chapter 4 to describe whole colony behaviour require complementation with observations of individuals.

Two fundamental assumptions in the model concern the behaviour of individuals:

- there is a fixed 'sleep time' during which no individuals will become active. Hence we expect to observe a minimum relaxation period equivalent to s during which individuals remain inactive;
- all individuals are identical with respect to 'waking' and 'sleeping' be-

haviour. Hence we expect to observe no difference between castes such as Innendienst and Aussendienst with respect to these parameters.

If it assumed that some fixed relaxation period exists, then in order to account for the variability in observed colony activity cycle length, the probability of individuals waking spontaneously must be very low. If stimulation is physically mediated, we expect to observe activity onsets frequently following physical contact, and rarely occuring 'spontaneously'. If stimulation is chemically mediated, we cannot test this suggestion by observation alone.

These assumptions were tested by observation of individuals from video recordings of the nest. The use of video recordings allowed behaviours to be measured simultaneously for different individuals, and direct comparison of parameters for individuals to those for the whole nest (Chapter 4). The colony was filmed in the constant temperature room under the same conditions as those in which digitized image analysis was conducted. The film was recorded on the day following conclusion of image analysis; hence it seems reasonable to assume that the filmed sample is representative of the behaviour of individuals whilst image analysis was conducted.

5.2 Methods

Time lapse video recordings of the colonies used in the digitization runs discussed in the previous section were made using a Panasonic NV-8050 timelapse recorder attached to a monochrome video camera (Panasonic model WV-1850/B with Fujinon TV zoom lens), onto TDK HS E-180 VHS tapes. Recordings were made at 16× real time. Each colony was filmed for 7 hours on the day following

the last day of the digitization run; the colony was not moved from its previous position on the light box during this time. Filming commenced at 11 am, as with the digitization runs. Hence each colony was filmed under conditions as similar as possible to those in which image analysis experiments were conducted. The timelapse recorder inscribed a time signal on the tape allowing the time at which various events occured to be read directly from the tape on replay.

The activity of individuals was followed from the point corresponding to 11.30 am, the first 30 minutes of tape were ignored to reduce the likelihood that ants were responding to disturbance when filming commenced.

In order to investigate differences between individuals, two groups from each nest were studied: those that occupied positions on or near the brood pile (refered to as 'brood workers') and those that clustered around the exit of the nest (which I refer to as 'doorhangers'). These two groups were expected to correspond to the groups Innendienst and Aussendienst distinguished in Section 2.2. The resolution of image was generally not sufficient to allow clear recognition of queens, so I was unable to study these individuals as a separate group.

For each colony, 5 individuals were chosen at random from each group. Their position was marked at the start time (11.30 am) on an acetate sheet to avoid choosing the same individual again. Where possible, I followed each individual through 10 consecutive phases of activity and inactivity. Individuals were defined to be active if they moved around the nest or were performing acts such as grooming without locomotion. The ants were described as inactive if they were stationary. Occassionally, ants left the nest before 10 sets of observations were completed; in these cases I followed further individuals to maintain the sample size. The further individuals chosen were the first ant (not already studied) to

enter the nest after the start of the observation period. This regime allowed inclusion in the 'doorhanger' group of ants that enter and leave the nest as well as ants that congregate by the exit but do not leave the nest.

I recorded the following data for each individual:

1. the time at which each 'sleep' and 'wake' phase commenced;
2. whether the individual became active spontaneously or received contact prior to waking (see below);
3. the number of times the ant received contact during each 'sleep' phase. In some cases I also recorded the times at which these contacts occurred, for comparison to work conducted by Cole (1991b).

Clarification of terms

For brevity and clarity, I have referred to ants being in the 'wake' and 'sleep' phases, by which I mean 'inactive' and 'active' respectively. I do not mean to suggest that there is any similarity other than by analogy to sleep and wake in higher animals.

I have also referred to individuals 'receiving contacts': by this I mean that the focal animal received physical contact from another individual. Physical contact may result from 'casual bodily contact' (Section 2.1.1), receiving antennations or grooming from an active individual.

I describe an ant as 'waking spontaneously' if it does not receive contact imme-

diately before becoming active. This linkage of events as preceeding or following 'immediately' is clearly subjective. From initial measurements however, individuals appeared to wake within 1 second of contact, or did not appear to respond at all. Spontaneous wakings were clear as the focal individual became active whilst others in the vicinity were still inactive. The linkage of 'contact' and 'waking' may have no causal relationship; the ant's perceived time scale might be quite different to my own. Also, ants that wake 'spontaneously' may be responding to a contact that occurred some time previously. It is also true that individuals may respond to a stimulation that I did not record, for example the fast movement across their visual field of an individual that does not make physical contact.

5.3 Results

Fixed sleep time

If the assumption of fixed sleep times were correct, we would expect log survivorship curves of sleep duration to consist of a horizontal shoulder followed by a linear decay. For *L. acervorum* broodworkers, this appears to be the case (Figure 5.1a, c). *L. acervorum* doorhangers similarly exhibit a linear decay in log survivorship of sleep duration, although the horizontal shoulder is of rather shorter duration (Figure 5.1b, d). Data from different individuals and nests has been amalgamated on the assumption that individuals are identical within task group with respect to activity parameters, and that parameters are independent of particular colony. The distribution of sleep duration for *L. tubero-interruptus* workers (Figure 5.1e, f) also depart from the expectations, in that the decay is less clearly linear, and the horizontal shoulder is curtailed particularly for doorhangers.

By visual inspection, I obtained an estimate of s by fitting lines conforming to a shoulder and a linear decay to the distributions for each task group in each nest (Appendix D.5). The estimates (Table 5.3.1) for both doorhangers and broodworkers are consistently lower than those obtained in Chapter 4 from digitized data for the whole colonies. The actual minimum sleep bout length from individual measurements clearly indicates that the minimum duration is not fixed: for *L. acervorum* broodworkers, a minimum duration of 143 seconds was observed, and one of 26 seconds observed for doorhangers. The minimum observed durations were similar for *L. tubero-interruptus*, where 112 seconds was observed for broodworkers and 86 seconds for doorhangers.

Table 5.3.1

Nest	Log Estimate BW	Log Estimate DH	Model Estimate	Actual BW	Actual DH
BIGRUN	508	164	912	258	79
MIDRUN	557	115	1043	257	26
SMARUN	344	230	982	261	84
LITRUN	459	131	1044	143	38
DIFRUN	295	180	1129	204	86
TUBRUN	546	137	1185	112	124

Estimates of base sleep time s (in seconds) from inspection of log survivorship distributions of bout length of individuals. The second and third columns show s estimated for brood workers and door hangers respectively. The estimate for each run from the WSCCS model and digitized data is shown in the fourth column. The observed minimum sleep time for brood workers and door hangers is given in columns five and six.

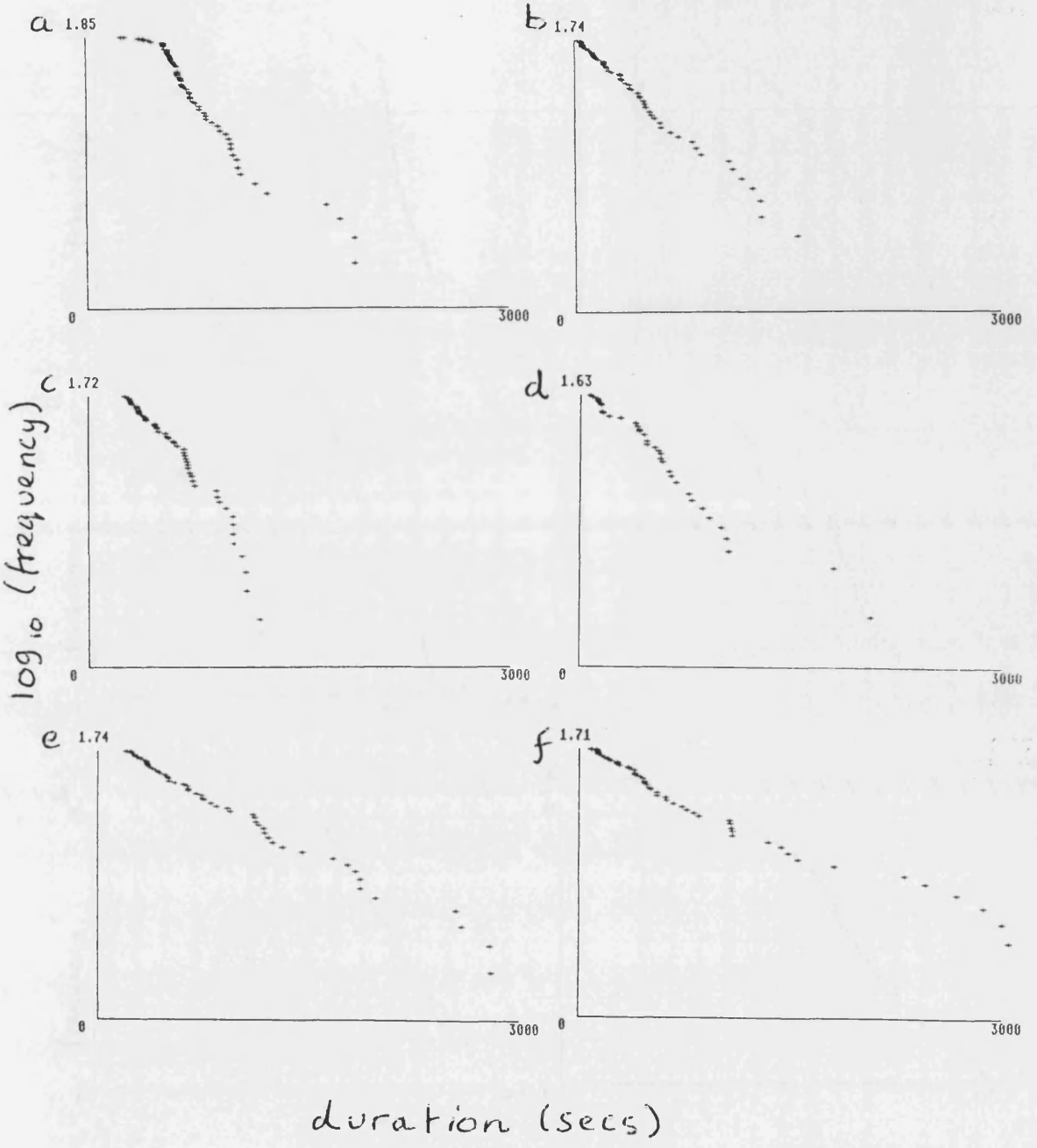

Figure 5.1: Log survivorship of inactive phase duration (in seconds) of individual ants. Each point shows the number of observed events longer than the interval shown on the horizontal axis. MIDRUN:a, brood workers; b, door hangers. SMARUN:c, brood workers; d, door hangers. DIFRUN: e, brood workers; f, door hangers.

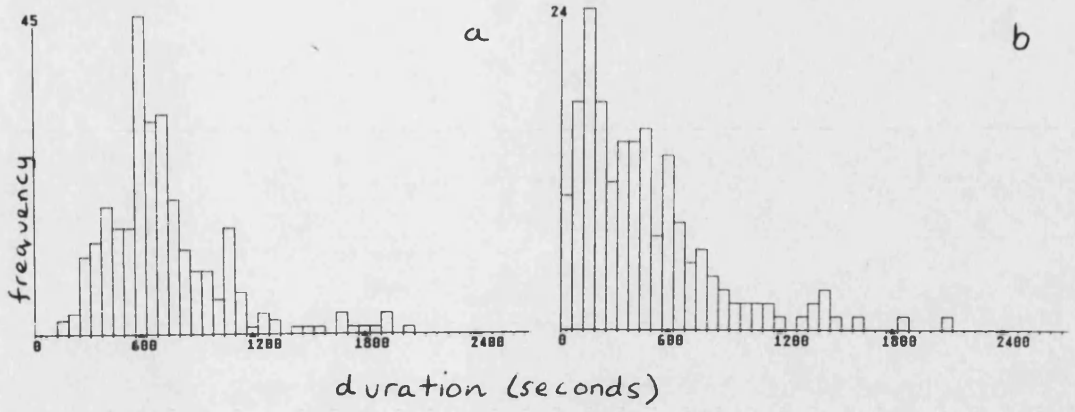

Figure 5.2: Distribution of inactive phase length (in seconds) for *L. acervorum* individuals. a, brood workers; b, door hangers. Distributions show data amalgamated from BIGRUN, LITRUN, MIDRUN and SMARUN.

Identical individuals

Student's T test was employed to compare the distribution of mean bout lengths of broodworkers and doorhangers for both species. Figure 5.3 summarizes the 95% confidence intervals for sleep bout duration. For *L. acervorum*, differences between broodworkers in different nests, or between doorhangers in different nests, were not significant. However, doorhangers tend to possess a shorter sleep bout duration than broodworkers; this difference is significant ($P<0.05$) for two colonies (BIGRUN, LITRUN). *L. tubero-interruptus* workers tend towards longer sleep bout durations than *L. acervorum* workers (not significant, with the exception of TUBBW), but there is no clear evidence that the task groups differ with respect to sleep duration in this species.

In 3 out of 4 cases, *L. acervorum* broodworkers remain active significantly longer than doorhangers ($P<0.05$; Figure 5.4). *L. tubero-interruptus* workers do not appear to differ significantly in wake bout duration compared to *L. acervorum* workers, nor is there any evidence that doorhangers and broodworkers of this species differ significantly with respect to this parameter. Mean active bout du-

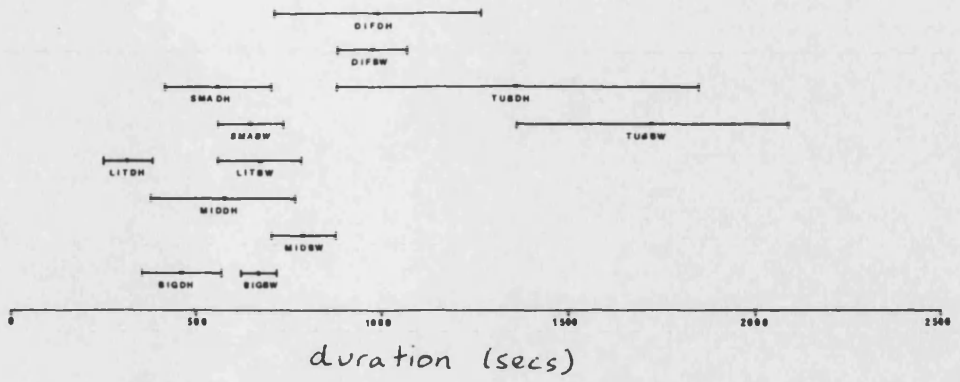

Figure 5.3: 95% confidence intervals of inactive phase duration. For each run (as marked), for brood workers (BW) and door hangers (DH) the mean (in seconds), lower and upper confidence limits are shown.

ration is in the order of 3 minutes for both species, ranging from 108 seconds (SMADH) to 296 seconds (SMABW). In all cases, active bout duration is significantly shorter than sleep bout duration (comparison of 95% confidence intervals, Figures 5.4, 5.3, for given run and task group).

The mean duration of sleep time in *L. acervorum* workers appears to lie between 318 seconds (LITDH) and 790 seconds (MIDBW); that in *L. tubero-interruptus* between 976 seconds (DIFBW) and 1724 (TUBBW). The estimate of s from the WSCCS model, as measured from activity timeseries for whole colonies (Chapter 4) lies in the region of 873 seconds (BIGRUN) to 1044 seconds (LITRUN) for *L. acervorum* (Table 4.4.3). This estimate includes sleep and wake bout duration, since wake bouts are assumed to be arbitrarily small for simplicity. Hence a more appropriate comparison between whole colony and individual estimates might be that between s and wake plus sleep bout duration.

By the addition of the preceeding wake duration W to a given sleep duration S (for each measurment of W and following S, for each individual), I obtained an

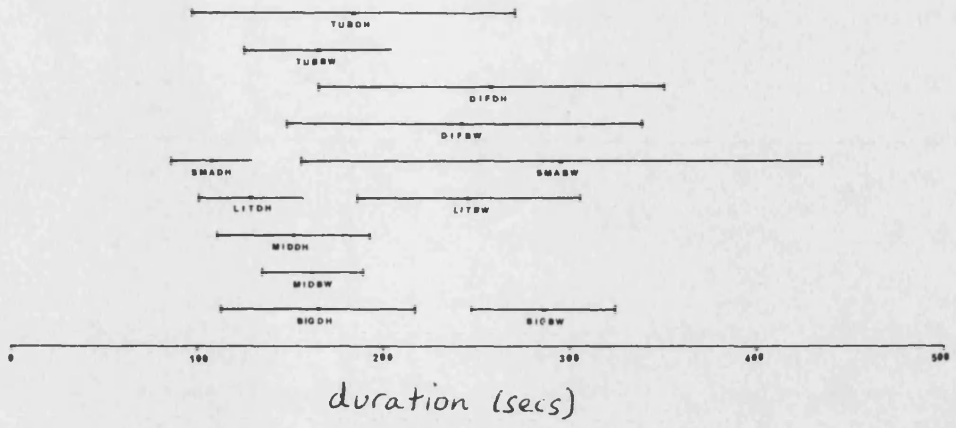

Figure 5.4: 95% confidence intervals of active phase duration. For each run (as marked), for brood workers (BW) and door hangers (DH) the mean (in seconds), lower and upper confidence limits are shown.

estimate of mean cycle duration W+S for individuals. Figure 5.5 summarizes the 95% confidence intervals of mean W+S distributions, and comparison to mean cycle length and s from the whole colony data presented in Table 4.4.4. For *L. acervorum*, in 2 out of 4 cases doorhangers appear to possess significantly ($P<0.05$) shorter activity cycles than brood workers; in the other two nests, the difference is in the same direction but is not significant. For *L. tubero-interruptus*, cycle length does not differ significantly between task groups, but may tend to be longer than that for *L. acervorum* (not significant).

For *L. acervorum*, in all cases the estimate of s (whole colony data) lies within the 95% confidence interval for broodworkers, but the mean colony cycle length is significantly longer. For *L. tubero-interruptus*, s lies within the 95% confidence interval of the task group with the shorter cycle duration, and the measured colony mean lies within the 95% confidence intervals of the task group with the longer cycle duration. Hence it appears that s estimates individual (brood worker) cycle length, and that colony level cycle means exceed this estimate.

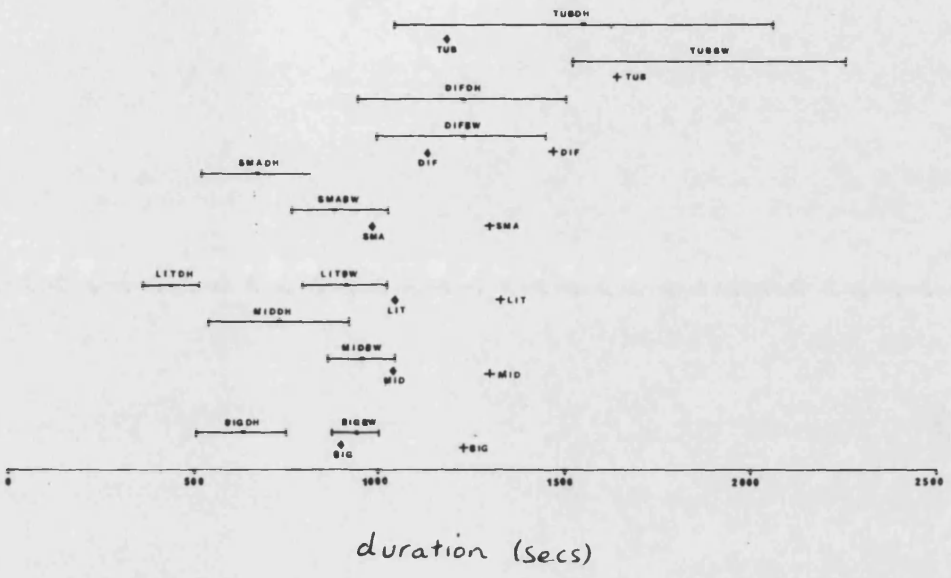

Figure 5.5: Mean cycle length and estimate of s from colony and individual data. For each run and task group (as marked), the mean and 95% confidence interval of cycle duration (from individual measures of active and following inactive phase) are shown. ◆ indicates estimate of s from colony data and WSCCS model. + indicates mean cycle length as measured from colony data presented in Chapter 4.

The results presented in this section also indicate that in the case of *L. acervorum* there is some evidence suggesting that task groups are not identical with respect to wake, sleep and cycle duration, but in *L. tubero-interruptus* workers do not appear to differ significantly in this respect.

Probability of Spontaneous Waking.

Tables 5.3.2, 5.3.3 present data concerning wake events that occur spontaneously or directly after receiving physical contact, respectively. For both species and both task groups, the proportion of spontaneous waking is approximately 0.1. Spontaneous waking does not occur at significantly different frequencies between species or task group (χ^2 heterogeneity test, P> 0.05). Hence, roughly 90% of all wakings occur within 1 second of physical contact. However, physical contact will not necessarily precipitate waking: the mean number of physical contacts received during a sleep bout (Figure 5.6) lies between 3.3 (TUBBW) and 5.8 (ACEDH). The number of physical contacts received during sleep bouts that terminate spontaneously is also nonzero, although there is a suggestion (especially in *L. tubero-interruptus*) that individuals waking spontaneously have received fewer contacts those those not waking spontaneously in the preceeding sleep bout.

Table 5.3.2

Task	*n*	$\overline{x}$	*Sd*($\overline{x}$)	$\overline{h}$	*Sd*($\overline{h}$)	*min sleep*	*max sleep*
ACEBW	*37*	*696.89*	*224.55*	*2.68*	*2.90*	*223*	*1221*
ACEDH	*21*	*540.29*	*505.32*	*2.62*	*3.43*	*59*	*2077*
TUBBW	*10*	*1333.10*	*1089.36*	*0.6*	*1.26*	*235*	*2806*
TUBDH	*10*	*826.90*	*761.89*	*0.4*	*0.84*	*181*	*2681*

Data concerning spontaneous waking events. For each species and task group (ACE, L. acervorum; TUB, L. tubero-interruptus; BW, brood workers; DH, door hangers), the number (n) of observed spontaneous waking events is given. The mean ($\bar{x}$; in seconds) and standard deviation ($Sd(\bar{x})$) of inactive phases preceding spontaneous waking events is given and also the mean number ($\bar{h}$) and standard deviation ($Sd(\bar{h})$) of contacts per such sleep phase. min sleep and max sleep refer to the minimum and maximum duration (in seconds) of inactive phases observed preceding spontaneous activation.

Table 5.3.3

Task	*n*	$\bar{x}$	$Sd(\bar{x})$	$\bar{h}$	$Sd(\bar{h})$	*min sleep*	*max sleep*
ACEBW	*240*	*696.98*	*335.69*	*3.16*	*2.36*	*143*	*1985*
ACEDH	*168*	*470.96*	*484.06*	*5.81*	*4.95*	*26*	*3831*
TUBBW	*95*	*1331.73*	*1085.36*	*3.32*	*2.28*	*112*	*5050*
TUBDH	*83*	*1198.10*	*1313.50*	*4.16*	*3.43*	*49*	*8532*

Data concerning contact driven waking events. n, number of observed contact driven events. The mean ($\bar{x}$; in seconds) and standard deviation ($Sd(\bar{x})$) of inactive phases preceding contact driven waking events is given and also the mean number ($\bar{h}$) and standard deviation ($Sd(\bar{h})$) of contacts per such sleep phase. See also Table 5.3.2

The frequency distribution of contacts per sleep bout (Figure 5.7) does not suggest that waking is precipitated by receiving some threshold number of contacts. Although the modal contact frequency lies between 1 and 3 per sleep bout for *L. acervorum* there is a clear spread, in one case 39 contacts were received before the bout terminated. In the case of *L.acervorum* there is a suggestion that the frequency distribution of contacts is more dispersed for DH than BW. This is

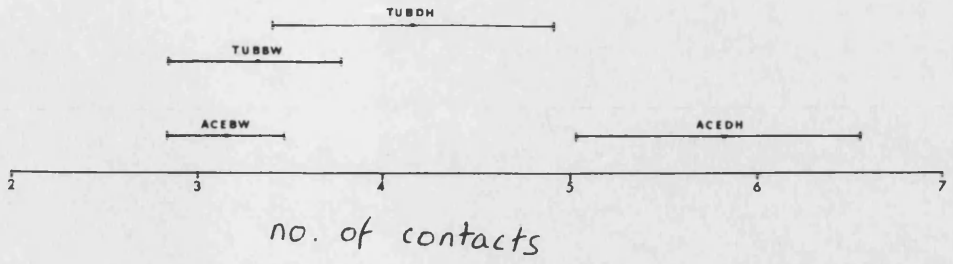

Figure 5.6: 95% confidence intervals of the mean number of contacts per inactive phase. The mean and lower and upper confidence limits (from Students T test comparison) for each species and task group are shown. ACE, *L. acervorum*; TUB, *L. tubero-interruptus*; BW, brood workers; DH, door hangers.

supported by Student's T test (Figure 5.6) for *L. acervorum*, broodworkers receive significantly fewer contacts per sleep bout than the door hangers ($P < 0.05$). As with bout length data, the *L. tubero-interruptus* task groups do not differ significantly.

Hence from the data concerning frequency of contacts per sleep bout, there is further evidence that *L. acervorum* individuals are not identical with respect to activity parameters. Also, spontaneous waking is relatively uncommon in both species and task groups. Receipt of contact is not always followed by waking, neither is there evidence for a threshold in contacts that precipitate activity.

5.4 Discussion

Spontaneous waking

The results indicate that spontaneous waking is relatively rare, occuring in approximately 10% of cases. This might suggest that the waking signal is not mediated by pheromones, since chemical based stimulation might imply more

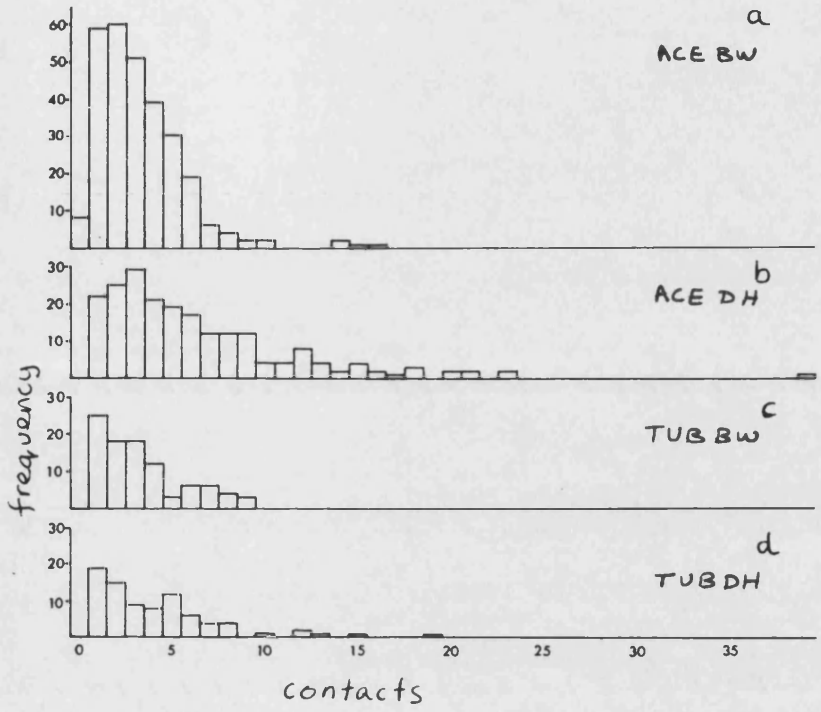

Figure 5.7: Frequency distribution of contacts per inactive bout. For species and task groups the frequency of occurrence (vertical axis) of a given number of contacts per inactive bout (horizontal axis) is shown. ACE, *L. acervorum*; TUB, *L. tubero-interruptus*; BW, brood workers; DH, door hangers.

'spontaneous' waking events (as opposed to those that appear to result from physical contact).

That a given physical contact is certainly not always followed immediately by waking is consistent with the mechanism underlying Tofts' model (1990a). Failure to wake may result from the focal individual still being unresponsive to the signal at that point (still in sleep phase). Also, lack of evidence for a threshold number of contacts after which waking occurs supports the hypothesis that individuals are responding to intrinsic timing factors (e.g., minimum sleep time) rather than externally mediated features in time.

Individual differences

The assumption that individuals are identical with respect to activity parameters is shown to be an oversimplification in the case of *L. acervorum*. In this species, there is evidence that individuals involved in brood care possess longer bouts of activity ($P < 0.05$ in 2 out of 4 cases) and inactivity (3 out of 4 cases) than individuals that reside near the entrance, resulting in a longer wake-sleep cycle length for broodworkers (2 out of 4 cases). Also, individuals not involved in brood care appear to receive more physical contact per bout of inactivity than brood workers, suggesting that they are less likely to respond to instances of contact than brood workers ($P < 0.05$). The task groups do not appear to differ in the frequency of wakings that follow physical contact ($P > 0.05$). In *L. tubero-interruptus*, no significant difference was found between the two task groups with respect to these parameters, indicating that the assumption of identity may be acceptable for this species.

Fixed sleep time

Measurements of individual sleep bout length do not support the assumption of a fixed minimum bout length s in the order of 900 seconds (as estimated in Section 4.4.3 for *L. acervorum*). Individuals near the entrance may wake within 1 minute of the onset of sleep; broodworkers may wake within 2 minutes, although the modal sleep bout length is in the order of 8 to 10 minutes respectively (for *L. acervorum*).

A more complicated version of the WSCCS model (Tofts, 1990a) incorporates a variable sleep period, by proposing that inactivity is divided into two phases (Figure 5.8). During the initial phase, individuals do not wake spontaneously or as a result of stimulation (this phase is identical to the minimum sleep phase s in the former model). Following this, individuals enter a responsive sleep phase s', during which they may wake as a result of stimulation, but will not wake spontaneously. Following this phase, individuals behave as before; they may wake spontaneously or will wake if stimulated by another.

Under such a model, one would expect to observe a minimum period s' during which no spontaneous wakings occur, and a shorter interval s during which waking does not occur at all. After the period s' has been exceeded, we would expect waking to occur spontaneously or follow the next stimulation. Unfortunately, a direct test of these predictions is not possible from the data collected so far. The distribution of sleep bout lengths preceeding spontaneous waking does not differ significantly from that preceeding stimulated waking, but for each task group the observed minimum was greater in the former case. Further collection of data on individual parameters, especially whether the probability of waking after a stimulation approaches unity after fixed interval s' has elapsed, may clarify this

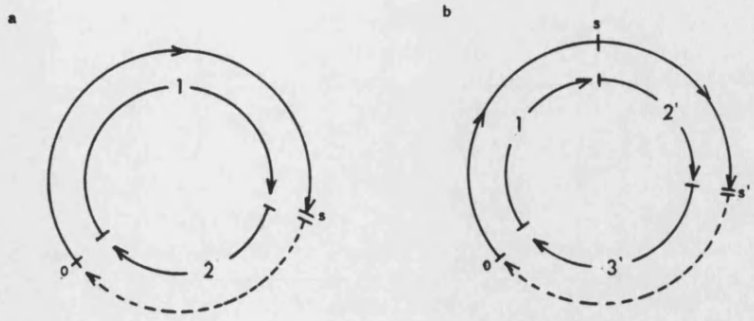

Figure 5.8: Diagrammatic representation of WSCCS models. a, WSCCS model 1. During phase 1 (inner circle), an ant is in the sleep phase; it is not responsive to contacts and does not wake spontaneously. Phase 1 lasts from time 0 to s (outer circle). During phase 2 the ant is wakeable; it may wake spontaneously and will respond to contact. The phase is of variable duration, the active phase itself is assumed to be part of the sleep phase. b, WSCCS model 2. Phase 1 as above. Phase 2' is the responsive sleep phase; the ant may respond to contact but does not wake spontaneously. Phase 3' is equivalent to phase 2 above.

issue.

The distribution of cycle length at the colony level under the revised model is complicated, since positive feedback will not neccessarily occur after the first individual wakes spontaneously (at s plus some probabilistic element). Instead, we would expect a near geometric decay after time s' convoluted with some distribution between s and s' (Figure 5.8b). The observed distributions (Section 4.3.6) are similar to this expectation, and could be fitted to such by appropriate adjustment of parameters. However, without biological justification for the values chosen for parameters (on the basis of estimates from individuals for probability of waking, s' and s), such an endeavor is unlikely to reward us with understanding of the mechanism involved.

The revised model contains two interesting properties not possessed by the simple version. Firstly, the division of inactivity into two phases allows inclusion

of nonidentical individuals with relative ease. Differences in the activity parameters of individuals can be incorporated by manipulating the relative durations of deep and responsive sleep periods, rather than including new elements with structurally different properties.

Secondly, it can be shown that assemblages of individuals with biphasal inactivity are less prone to error in overall cyclicity than those with unitary phases, if the elements themselves are prone to error (Tofts 1990a). Hence, if synchronized activity were to be advantageous (see Chapter 8) it may have been easier to achieve via selection for biphasal individuals, rather than for uniphasal individuals with perfect timekeeping.

Individual and colony level measures

The mean cycle length for individuals (wake plus sleep bout lengths) appears to be shorter than that for the whole colony (from digitized data; Chapter 4) by a factor of several minutes in *L. acervorum*. Although estimated minimum cycle time (s) lies within 95% confidence intervals of mean $W + S$ bout length for brood workers, mean colony cycle length does not. Two possible explanations may account for this.

Firstly, the time lost between individual and colony level estimates may represent signal propagation time across the whole nest; the time required for the whole colony to respond to activity onset may exceed that for focal individuals. Secondly, cycles may be 'missed' in the colony data, leading to overestimation of cycle length. It appears from the data presented in this chapter that colony cycles consist of 2 or more oscillators with different frequencies (broodworkers

and doorhangers) coupled to a greater of lesser extent, in the case of *L. acervorum.* The interaction of two or more waves of activity may lead to phases of reinforcement or cancellation. Some troughs of activity in the brood pile may coincidence with peaks at the entrance, and consequently be missed in the data for overall colony activity. This suggestion is supported by some of the time series presented in Chapter 4 which appear to exhibit beating, that is, alternate regions of increased and reduced amplitude. Further, the occurence of short cycles (in the order of 11 minutes), as evidenced by some time series and their correlograms may indicate short cycling by door hangers. In these cases door hangers do not appear to be strongly coupled to brood workers, and their pattern of activity may obscure cycles on the brood pile from the automated measure of overall activity.

Chapter 6

The Effect Of Starvation On Colony Activity Cycles

6.1 Introduction

In Chapter 4, I attempted to test the prediction of Hemerik et al. (1990) concerning cycle length and brood to worker ratio. In retrospect, this prediction is difficult to test since it is not clear what precise measurement of brood to worker ratio should be employed: we do not know the relative rates of energy consumption of the various brood stages.

A more viable test of the model might be in relation to food deprivation. Hemerik et al. (1990) predict that as starvation proceeds the following behaviour should be observed:

- a decrease in cycle length;
- an increase in overall colony activity level;
- eventual breakdown of cycles, with a constant high level of activity.

6.2 Methods

6.2.1 Image Analysis

The techniques described in Chapter 4 were employed to measure activity of two *L. acervorum* colonies, starved over a period of at least 3 weeks (see Table 6.2.1). In each case, the colony was placed in the constant temperature room as described in Section 3.4.1 and allowed to acclimatize for 48 hours. Thereafter, image analysis proceded as described in Section 4.2; approximately 7 hours (416 frames) were snatched at 1 min intervals, commencing 11 am daily. For the first 7 days, forage was available to the colonies as described in Section 4.2; 1 M sucrose solution *ad libitum*, and *Drosophila* larvae replaced on the zeroth and third evenings after filming, when water was replenished. At the end of the 7th day, the sucrose solution was removed and no further *Drosophila* larvae added, so that no further food was available. On subsequent evenings the nest was inspected for signs that workers were consuming (a frequent occurence in social insect colonies deprived of sufficient forage; Nonacs, 1991) brood items, and water was replaced as neccessary.

Table 6.2.1

Run Name	*Start Date*	*End Date*	*W*	*Q*	*E*	*S*	*Me*	*L*	*P*	*M*	*D(S)*
STA	*20/2/91*	*3/4/91*	*113*	*5*	*90*	*56*	*59*	*28*	*3*	*0*	*22*
2ST	*30/5/91*	*15/7/91*	*115*	*3*	*11*	*21*	*37*	*6*	*0*	*0*	*24*

Summary of food deprivation experiments. The start date given is the day on which the first digitized recording was made. Colony census data indicate the number of items in the following categories: W, workers; Q, queens; E, eggs.

Larvae as defined in Chapter 2: S, small; Me, medium; L, large; P, pupae. M, males. D(S) indicates the number of days during which the nest was deprived of food sources.

When workers were observed to consume brood, a tube of 1 M sucrose solution was replaced in the petri dish just prior to the start of digitization on the following morning. On this day, image analysis commenced as usual, and I recorded various observations concerning discovery of and visits to the sucrose tube as presented in Section 6.3.3.

Image analysis was continued for at least 7 days after the return of food (during which time forage was replaced as for the first 7 days) in order to ascertain the recovery of the colony.

Digitized images were analysed as described in Section 4.2; mismatches were counted for the whole field of view and 9 separate windows (see Figure 4.1, Table 4.2.3) after the pixels had been grouped into 4 $\times$ 4 blocks and false colouring employed (see Table 4.2.2 and Table 3.4.1 for details). The time series of pixel mismatches were analysed as described in Chapter 4 and Section 6.3. below.

6.2.2 Observation of Individuals

Video recordings of the second nest (2ST) were made during a starved period, and 12 days after return of the food to the nest, using the methodology described in Chapter 5. The activity of individuals was measured by visual analysis of the video recordings as described in Section 6.3.5, to obtain information on the variation in individual parameters of activity under starvation. As in Chapter 5,

individuals were assigned to one of two groups, 'brood workers' (situated on or near the brood pile and observed to engage frequently in brood work), and 'door hangers' (situated near the entrance and not observed to engage in brood work). Where possible, individuals were followed for sufficient time to obtain ten of each of the following measures:

1. duration of sleep bout (S; measured as interval between onset of inactivity and next onset of activity);
2. duration of wake bout (W; measured as interval between onset of activity and following onset of inactivity);
3. duration of individual cycle W+S (measured as onset of active bout to onset of following active bout).

Also, the onset of a wake bout was scored as occuring either spontaneously or as directly following physical contact with another ant (see also Section 5.2).

6.3 Results

6.3.1 Timeseries: descriptive

Figures 6.1, 6.2 and Appendix D.1 depict raw daily time series data for the two starvation runs (Window X0: whole petri dish). Visual interpretation of these time series suggests that cycles of activity still occur in colonies undergoing prolonged starvation. Activity still appears to be cyclical on the last day of starvation in each case (22 and 24 days without food respectively; Figures 6.1c and 6.2c). This interpretation is supported by statistical analysis of turning

points (Table 6.3.1 and Table B.0.7; for each day, significantly fewer turning points occur in the raw data for this window than expected for a random series of the same length (95% confidence intervals; see Appendix Table A.0.6).

Table 6.3.1

Run Day	*N*	*TPs*	*LCI*	*UCI*
a STA01	*411*	*211**	*264*	*282*
b STA19	*408*	*201**	*262*	*280*
c STA29	*409*	*213**	*262*	*280*
d STA43	*411*	*212**	*264*	*282*
a 2ST01	*411*	*196**	*264*	*282*
b 2ST25	*411*	*185**	*264*	*282*
c 2ST35	*413*	*226**	*265*	*283*
d 2ST46	*405*	*226**	*260*	*278*

Turning point analysis of daily time series: samples. The number of idependent data points (N), observed number of turning points (TPs) for time series for window X0 are presented. For complete results, see Table B.0.7. a, food available; b, sample day whilst starving; c, last day of starving; d, after food was returned. The 95% confidence interval for random expectation is shown by LCI and UCI.

The results of autocorrelation are highly variable, as in Section 4.3.3. For both starved and fed days, correlograms exhibiting strong periodic components in the range of 10 to 20 minutes, and others showing no clear evidence of periodicity can be found (see Appendix D.3). First return maps (Figures 6.3) indicate no qualitative change in cyclicity in the time series from the whole petri dish under starvation (Figures 6.3 a, b), although cyclical activitity is less clear and substantially reduced in amplitude on the brood pile (Figures 6.3 c, d).

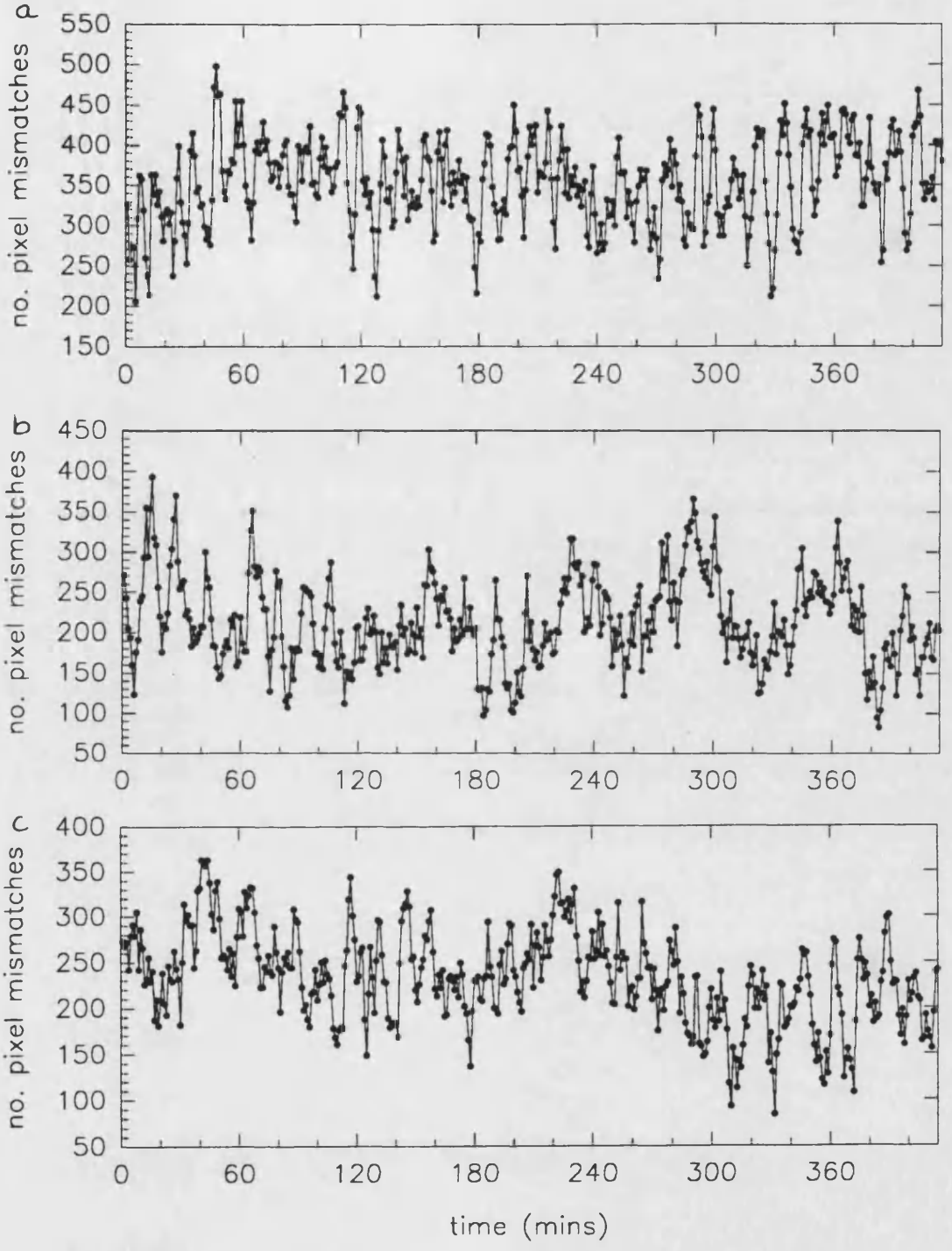

Figure 6.1: Sample time series of colony activity under food deprivation: STA, window X0. a, STA01 (food available); b, STA29 (food deprived for 22 days); c, STA31 (2 days after return of food).

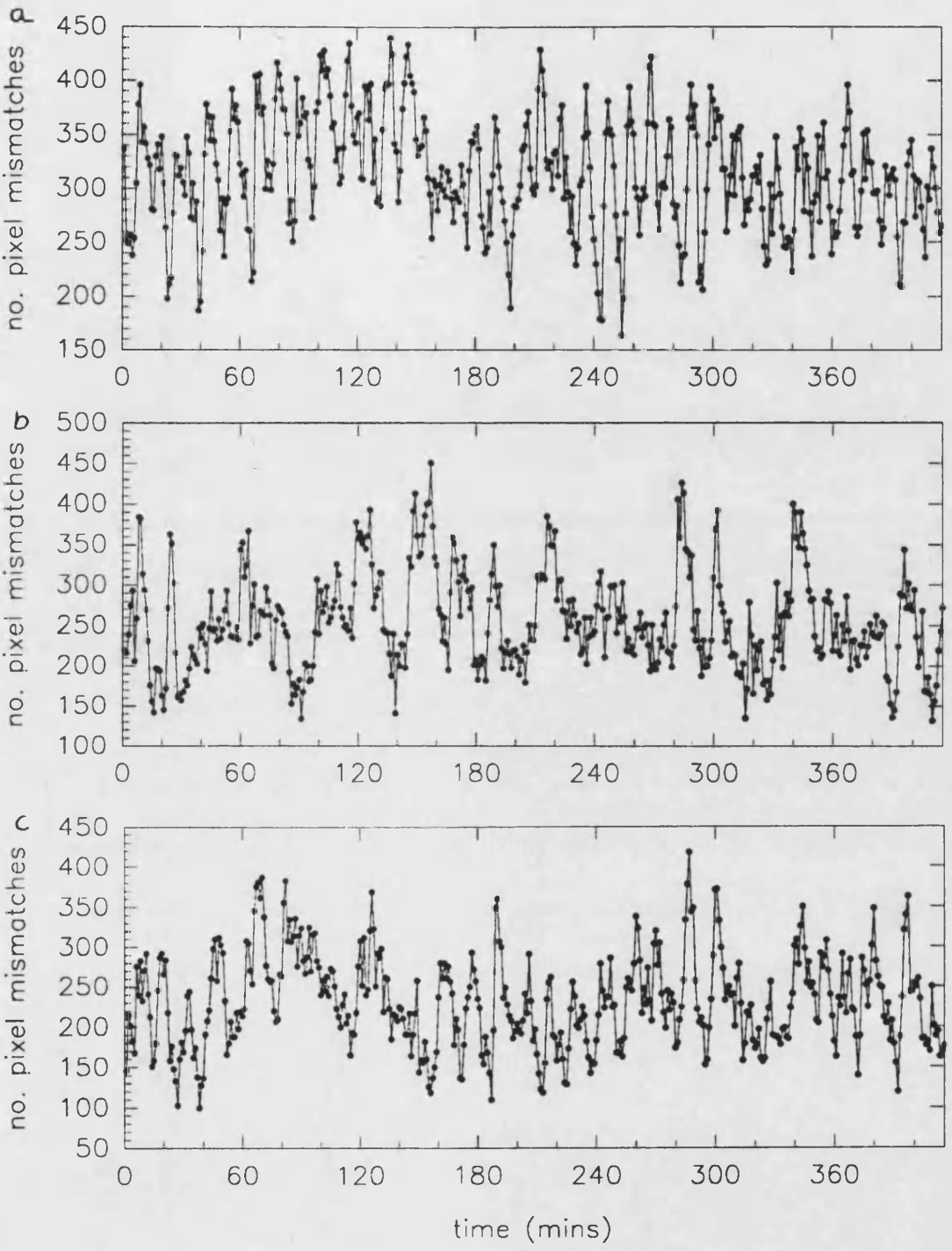

Figure 6.2: Sample time series of colony activity under food deprivation: 2ST, window X0. a, 2ST01 (food available); b, 2ST35 (food deprived for 24 days); c, 2ST38 (2 days after return of food).

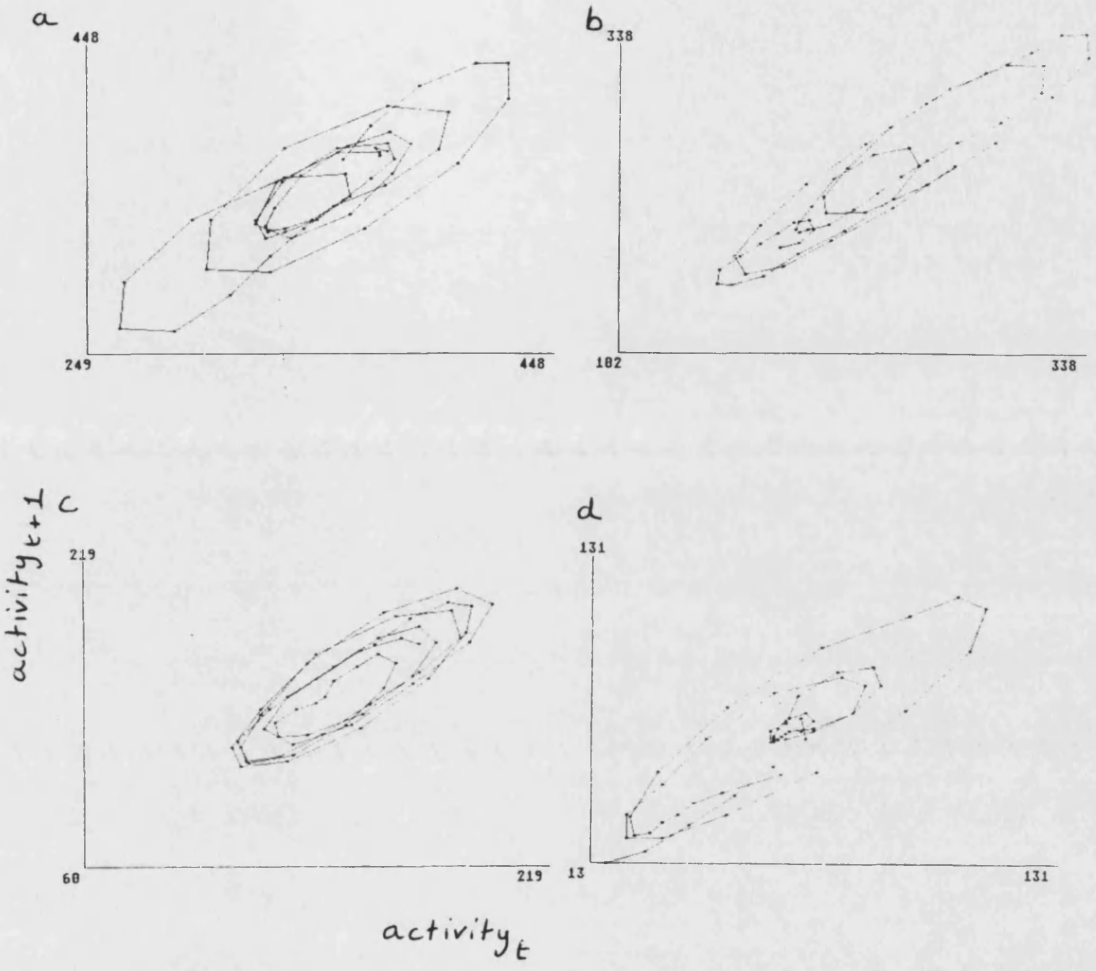

Figure 6.3: Sample return maps of activity in colonies under starvation. Activity at time t (horizontal axis) is plotted against activity at time $t+1$ (vertical axis; t in units of 1 minute). Window X0: a, STA01; b, STA29. Window 2: c, STA01; d, STA29.

6.3.2 Colony activity cycle length

The length of activity cycles was measured by the techniques described in Section 4.3.6: activity minima were located manually to obtain estimates of cycle length; these data were analysed to obtain estimates of mean and variance, and the distribution of cycle length compared to that expected from the WSCCS model (geometric distribution after a fixed lag s). To obtain sufficient data for these analyses the daily measurements were grouped into batches of consecutive days (see Table 6.3.2). For each experiment all days prior to food removal were grouped together, the period of starvation was divided into two sets of consecutive days, and all days after food return were grouped together. The day on which food was returned was not included in these analyses so as not to unduly bias the data: it is considered separately (Section 6.3.3 below).

Table 6.3.3 presents cycle length statistics for batches of days for both runs. There is some suggestion that cycle length decreases with increasing starvation (for both runs, mean cycle length appears slightly shorter for batches 2 and 3 compared to 1 and 4; see also Figure 6.4), although these differences are not significant ($P>0.05$; 95% confidence intervals: Table 6.3.2). In common with the data presented in Chapter 4, cycle length distributions are significantly right skewed ($P< 0.05$: positive g_1) and in some cases, significantly leptokurtic (positive g_2). Degree of skewness and leptokurtosis do not appear to be related to the level of starvation.

Table 6.3.2

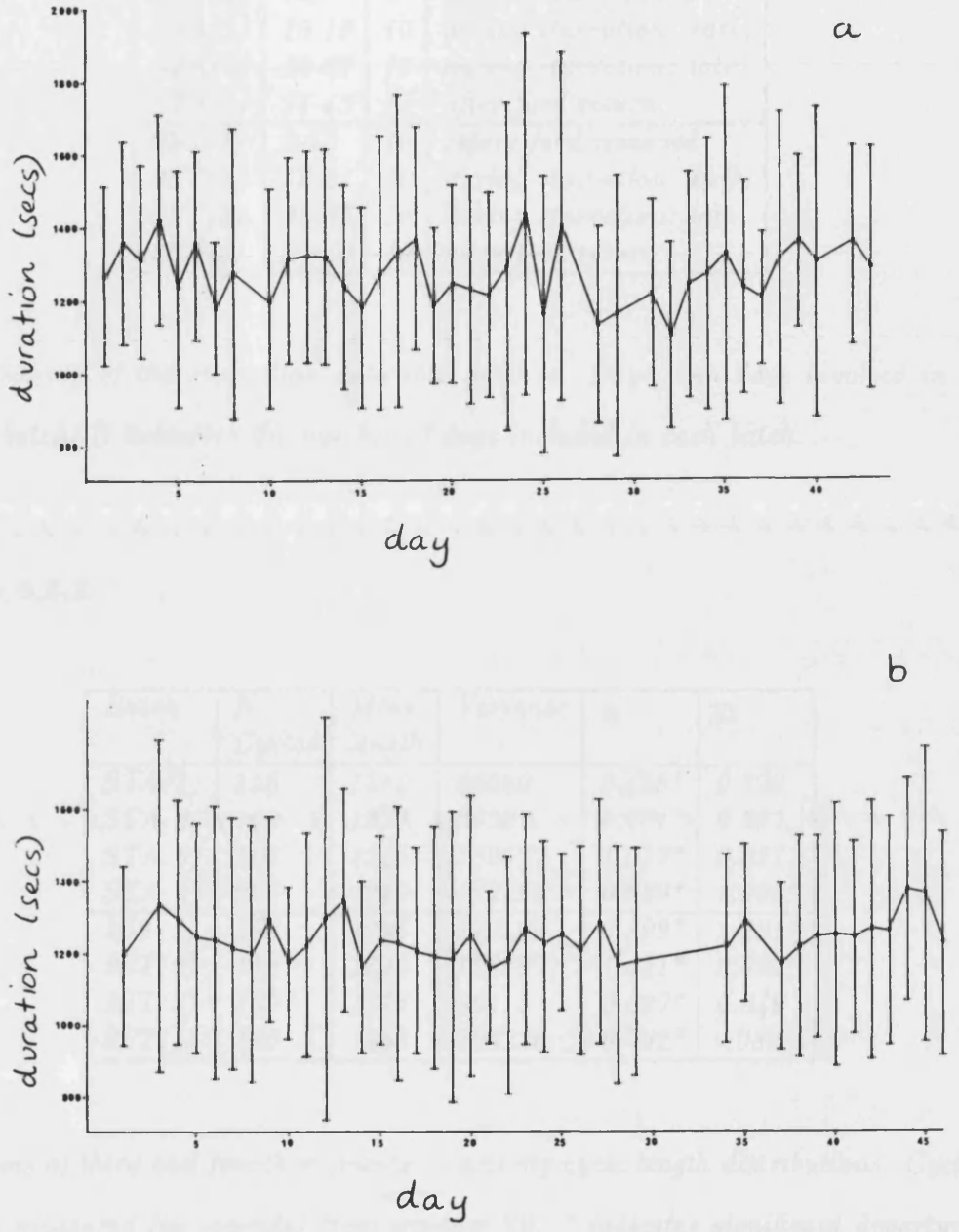

Figure 6.4: Mean and standard deviation of daily cycle length. a, STA; b, 2STA. Cycle length is measured using trough location applied to activity time series for window X0.

Batch	*Days*	*N*	*Notes*
STA(1)	*1-8*	*8*	*before food removed*
STA(2)	*10-19*	*10*	*during starvation: early*
STA(3)	*20-29*	*10*	*during starvation: late*
STA(4)	*31-43*	*12*	*after food return*
2ST(1)	*1-11*	*10*	*before food removed*
2ST(2)	*12-21*	*10*	*during starvation: early*
2ST(3)	*22-35*	*10*	*during starvation: late*
2ST(4)	*37-46*	*10*	*after food return*

Partitioning of the starvation data into batches. Days, run days involved in a given batch; N indicates the number of days included in each batch.

Table 6.3.3

Batch	*N Cycles*	*Mean length*	*Variance*	g_1	g_2
STA(1)	*158*	*1284*	*88680*	*0.458**	*0.399*
STA(2)	*162*	*1277*	*99337*	*0.761**	*0.297*
STA(3)	*166*	*1245*	*150577*	*1.013**	*0.621*
STA(4)	*206*	*1269*	*127482*	*0.989**	*1.005**
2ST(1)	*185*	*1235*	*112290*	*0.999**	*1.281**
2ST(2)	*186*	*1219*	*128897*	*1.451**	*3.721**
2ST(3)	*190*	*1218*	*86415*	*0.627**	*0.649*
2ST(4)	*180*	*1253*	*105336*	*0.792**	*-.032*

*Analysis of third and fourth moments in activity cycle length distributions. Cycle length measured (in seconds) from window X0. * indicates significant departure ($P< 0.05$) from normal expectation. Number of events (N Cycles), Mean cycle length and variance are given. g_1, third moment; g_2, fourth moment.*

Inspection of log survivorship curves (Figures 6.5a-h) also indicate no obvious differences in the distribution of cycle duration during periods when forage was (Figures 6.5 a,b,g,h) and was not (Figures 6.5 c-f) available. In common with

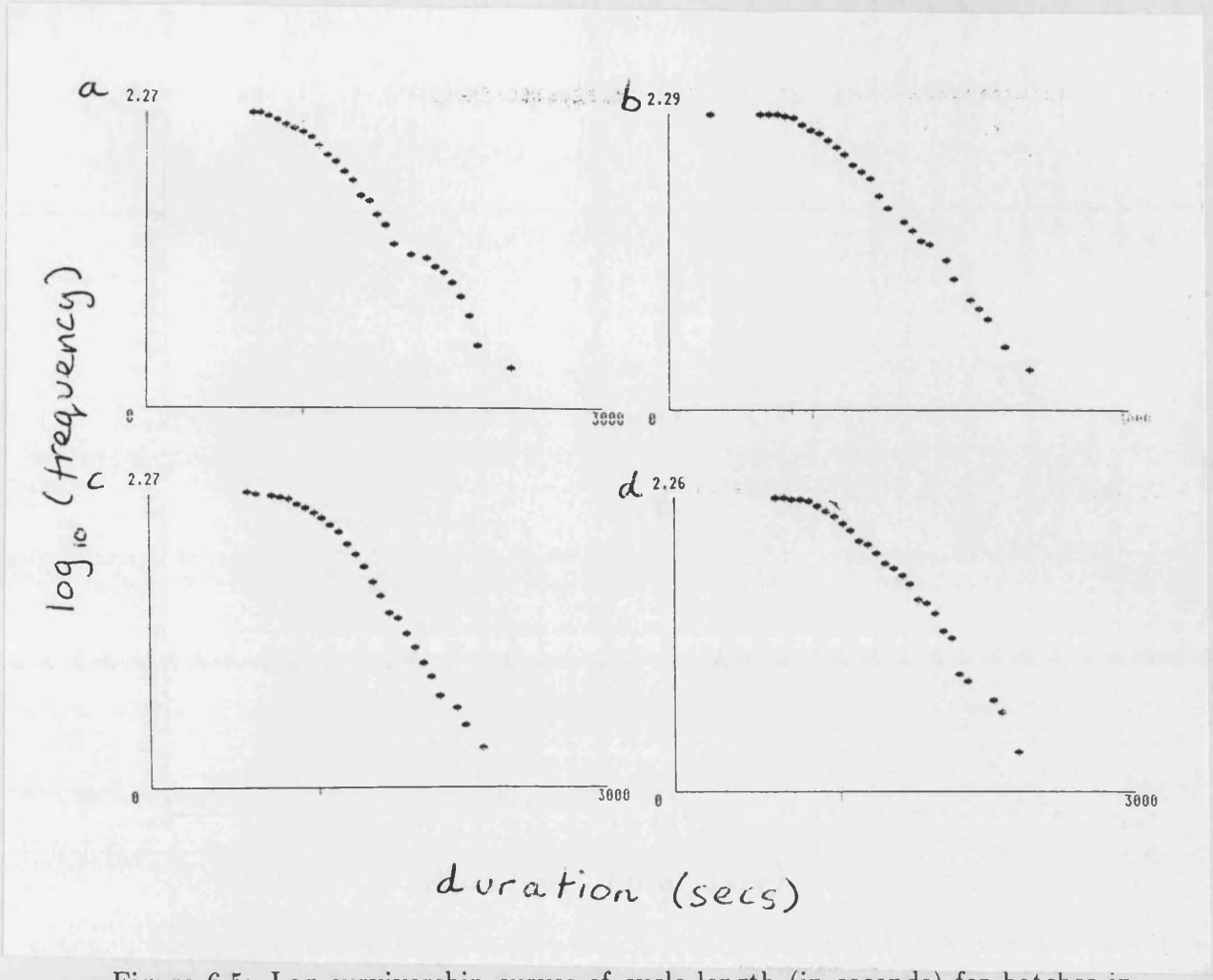

Figure 6.5: Log survivorship curves of cycle length (in seconds) for batches in the run 2ST. Each point shows the number of cycles that are longer than the duration shown on the horizontal axis. Batches: a, 2ST1; b, 2ST2; c, 2ST3; d, 2ST4.

the data presented in Section 4.3.6, these figures depict an extensive horizontal 'shoulder', followed by a roughly linear decay, supporting the prediction of Tofts that cycle lengths are distributed geometrically after a fixed lag. The intersection of lines 1 and 2 provides an estimate of minimum cycle length; in Table 6.3.4 these estimates are compared to those generated from the WSCCS model. As found in Section 4.4.3, the estimates from log survivorship generally coincide within the measurement error in the estimate of s.

Table 6.3.4

Batch	Log (S_l) Estimate	Model (S_g) Estimate	$S_l - S_g$
STA(1)	1024	985	39
STA(2)	975	961	14
STA(3)	918	857	61
STA(4)	909	912	-3
2ST(1)	926	899	27
2ST(2)	885	859	26
2ST(3)	998	924	74
2ST(4)	918	929	-11

Comparison of minimum cycle length estimates from log survivorship and geometric distribution. S_l: log survivorship estimates from intersection of lines fitted by inspection. S_g: estimate from WSCCS model and data. Cycle length data is from window X0 for each batch.

The results of the tests for goodness of fit of the observed cycle length data to distributions predicted by the WSCCS model are presented in Table 6.3.5. The data for starved nests fits the maximum likelihood distribution in 3 out of 4 cases, compared to 2 out of 4 cases when food was available (P> 0.05). In all cases, at least one permitted distribution from the nine generated for each batch did not depart significantly from that of the data (P> 0.05, for methods see Section 4.4.3 and Appendix A.4). The proportion of observed cycles that were shorter than the estimate of s does not appear to relate to degree of starvation, and is similar to that found in Chapter 4.

Table 6.3.5

Batch	*N*	*Mean length*	*s*	χ^2 *MLE*	χ^2 *Best*	*No.* χ^2 < 11.07	*No. Short cycles*
STA(1)	*158*	*1284*	*985*	*29.03*	*2.25**	*3*	*18*
STA(2)	*162*	*1277*	*961*	*9.7**	*6.13**	*2*	*14*
STA(3)	*166*	*1245*	*857*	*5.31**	*3.5**	*3*	*9*
STA(4)	*206*	*1269*	*912*	*9.77**	*6.61**	*2*	*15*
2ST(1)	*185*	*1235*	*899*	*14.55*	*1.63**	*3*	*21*
2ST(2)	*186*	*1219*	*859*	*8.94**	*6.07**	*4*	*11*
2ST(3)	*190*	*1218*	*924*	*14.27*	*9.63**	*3*	*21*
2ST(4)	*180*	*1253*	*929*	*10.46**	*6.54**	*2*	*8*

Summary of goodness of fit to WSCCS model. Cycle length (in seconds) measured from window X0. N, number of cycles observed. s calculated from data and model; for each batch nine test distributions were generated. The goodness of fit (χ^2 value) to the maximum likelihood distribution (χ^2 MLE) and to the distribution with best fit (χ^2 Best) are given. The number of distributions (out of nine) that did not depart significantly from the model expectation are also given (No. $\chi^2 <$ 11.07), and the number of cycles that were shorter than the estimate of sleep time s.

In summary, these results suggest that there may be a slight (but non significant) tendency towards decreasing cycle length with increasing food deprivation, but there is no clear evidence for a breakdown in cyclicity, or a qualitative change in cyclicity (cycle length distribution) under starvation.

6.3.3 Response to food return

Figure 6.6 a, b depict the raw time series data for colony activity level (Window X0) for the day on which food was returned to the deprived colonies. The figures indicate a clear sharp increase in colony activity which peaks within 1 hour of replacement of the sucrose tube. Also indicated in STA is an initially increased

activity level which may be the result of physical disturbance (replacement of food) just prior to the onset of digitization; it is clearly of little significance compared to the latter activity peak corresponding to the maximal colony response to food return. This peak in activity far exceeds any peaks measured as part of normal cyclical activity (Section 4.3 and Section 6.3.4). In the case of 2ST, frame grabbing did not commence until 11 minutes after the return of food, as a result of hardware malfunction.

At least during the peak phase of activity (i.e., approximately the first 2 hours of time series), the activity level does not appear to vary cyclically. As activity levels approach those normally found (200-350 pixel changes; see Figure 6.6), during the latter half of the time series, activity again appears to possess an element of cyclicity. On the following day activity has returned to more usual levels and is again cyclical (Appendix D.1).

Hence digitized activity measures suggest that colonies respond to food return with remarkable speed, and recover to normal levels and patterns of activity relatively quickly. This suggestion is supported by observational data (Table 6.3.7) and digitized data for separate portions of the nest (Figure 6.7). In both runs, within 5 minutes of the return of food, at least 1 individual discovered the food and returned to the nest. The number of foragers at the food source (instantaneous sampling) peaked within 30 minutes, as did the number of food exchanges within the nest. During this phase, solicitations of trophallaxis could be seen most frequently around the entrance and periphery of the brood pile towards the front of the nest. Replete foragers on return to the nest ran straight through the entrance and were frequently observed to drum their abdomen repeatedly against the floor of the nest. They were soon approached by a number of workers within the nest, which gathered round the forager, stroking her mandibles and baring

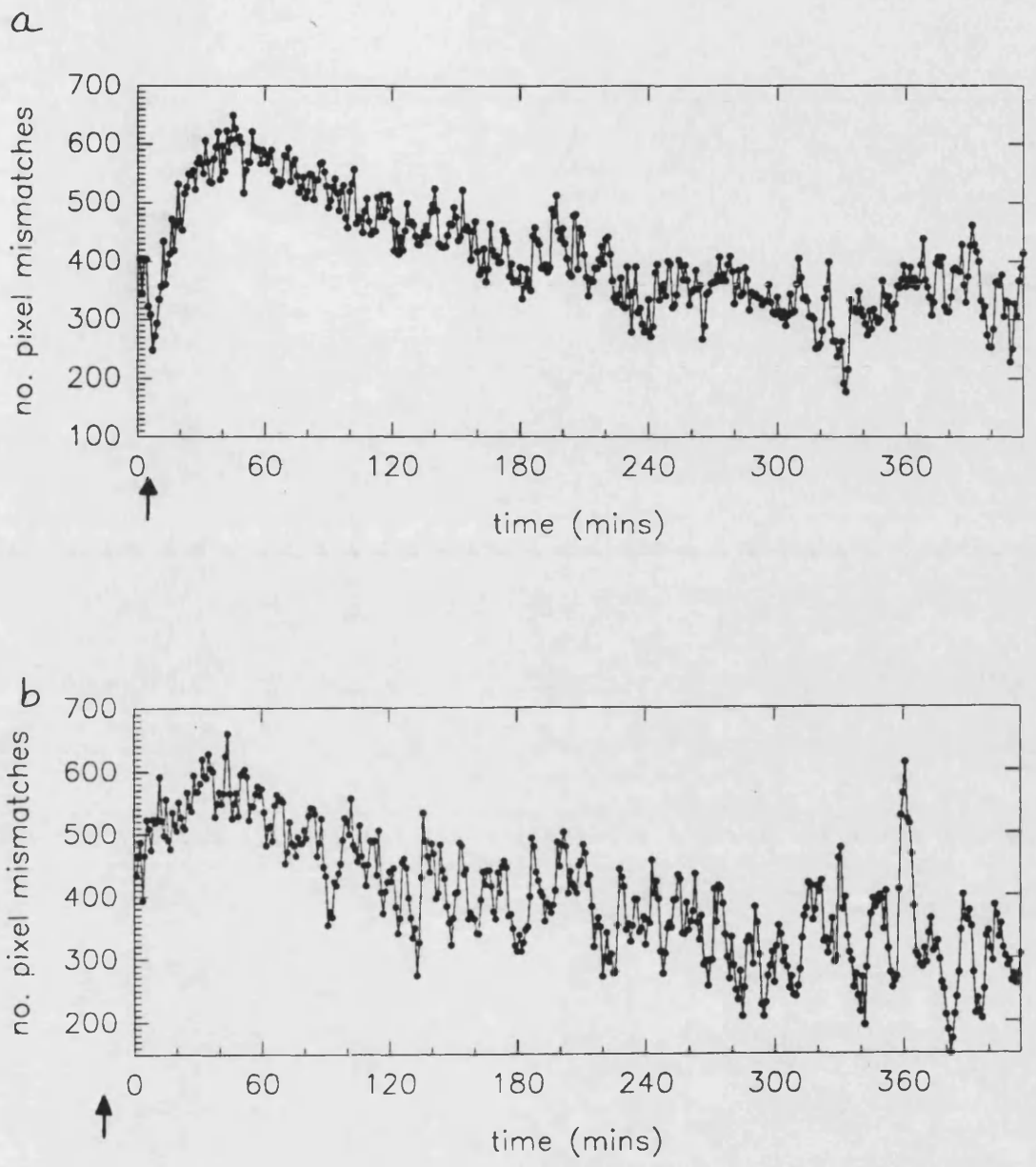

Figure 6.6: Time series of activity on the day of food return (window X0). An arrow on the horizontal axis marks the time at which food was replaced in each nest. a, STA30; b, 2ST36.

their own in food solicitation. These clusters of ants form quite striking 'rosettes' which are easily counted throughout the nest (Table 6.3.7).

Table 6.3.6

Run	*1st Ant on food*	*1st Ten on food*	*1st return*	*1st Ten return*	*1st Food exchange*
STARVE	*100*	*244*	*261*	*510*	*271*
2STARV	*47*	*159*	*294*	*320*	*300*

Observations on individual response to the return of food. Times are given in seconds relative to the return of food; for the first discovery of food, the first ten discoveries of food, the first return to the nest of a successful forager, the first ten such returns, and the first food exchange in the nest.

Table 6.3.7

Run	*Time*	*No. On Food*	*% out of Nest*	*No. Rosettes*	*% BP*	*% Periph*	*% Entr*
STA	*0*	*0*	*15*	*0*	*35(I)*	*20(I)*	*30(I)*
	10	*35*	*30*	*7*	*10(A)*	*30(A)*	*30(A)*
	20	*30*	*25*	*12*	*40(A)*	*25(A)*	*10(A)*
	30	*20*	*25*	*15*	*65(A)*	*5(A)*	*5(A)*
	60	*15*	*10*	*13*	*50(N)*	*30(A)*	*10 (A)*
	90	*15*	*10*	*6*	*45(N)*	*30(A)*	*15(A)*
	180	*5*	*5*	*4*	*50(N)*	*40(I)*	*5(I)*
2ST	*0*	*0*	*20*	*0*	*40(I)*	*10 (I)*	*30(I)*
	10	*18*	*20*	*8*	*1(I)*	*20 (A)*	*60 (A)*
	30	*21*	*25*	*14*	*70(A)*	*10(A)*	*5(A)*
	45	*11*	*20*	*13*	*60(A)*	*15 (A)*	*5(A)*
	60	*7*	*10*	*4*	*60(N)*	*20 (A)*	*10 (A)*
	90	*5*	*10*	*5*	*60 (N)*	*25(A)*	*5(I)*
	120	*5*	*10*	*4*	*55(N)*	*30(I)*	*5(I)*
	150	*7*	*10*	*2*	*55(N)*	*25(I)*	*5(I)*
	180	*4*	*5*	*2*	*55(N)*	*30 (I)*	*10(I)*

Response to food return. Values are given for various times (in minutes) after food return: the number of ants observed on the food, percent of colony out of the nest, the number of rosettes within the nest, percent of colony on brood pile, percent colony around periphery of brood pile, percent of ants at nest entrance. All observations by instantaneous count or estimate. Regions of the nest were also scored as being relatively inactive (I), active (A) or showing normal levels of activity (N; as in a fed nest).

Another phenomenon of interest from Table 6.3.7 is the spatial arrangement of food exchanges as the colony responds to the return of forage: initially (10-20 minutes) almost all workers leave the brood pile and congregate at its periphery close to the nest entrance, where most food exchanges occur. Within 30 minutes however, an increased number of individuals (relative to normal levels in well fed colonies) occur on the brood pile, and can be observed actively feeding brood items. Within 2 hours of food return, activity on the brood pile has returned to more normal levels, and some individuals were observed to be inactive on the periphery of the brood pile. By this point the number of food exchanges between adults has also reduced, and occurs in all parts of the nest (apart from directly within the brood pile). These observations are supported by the (digitized) measures of activity for various windows within the field of view: the activity level in the brood pile exhibits an initial decrease (10-20 minutes) followed by a peak at *circa* 40 minutes, corresponding to initial vacation of the area followed by increased activity as replete brood workers return and pass food on to the brood (Figure 6.7 a). Activity at the peripherary and entrance peaks at roughly 20 minutes and then at 60 minutes (Figure 6.7 b) and that outside the nest at 5 minutes and 55 minutes after food return (Figure 6.7 c).

We can summarize several points of interest from the data presented in this

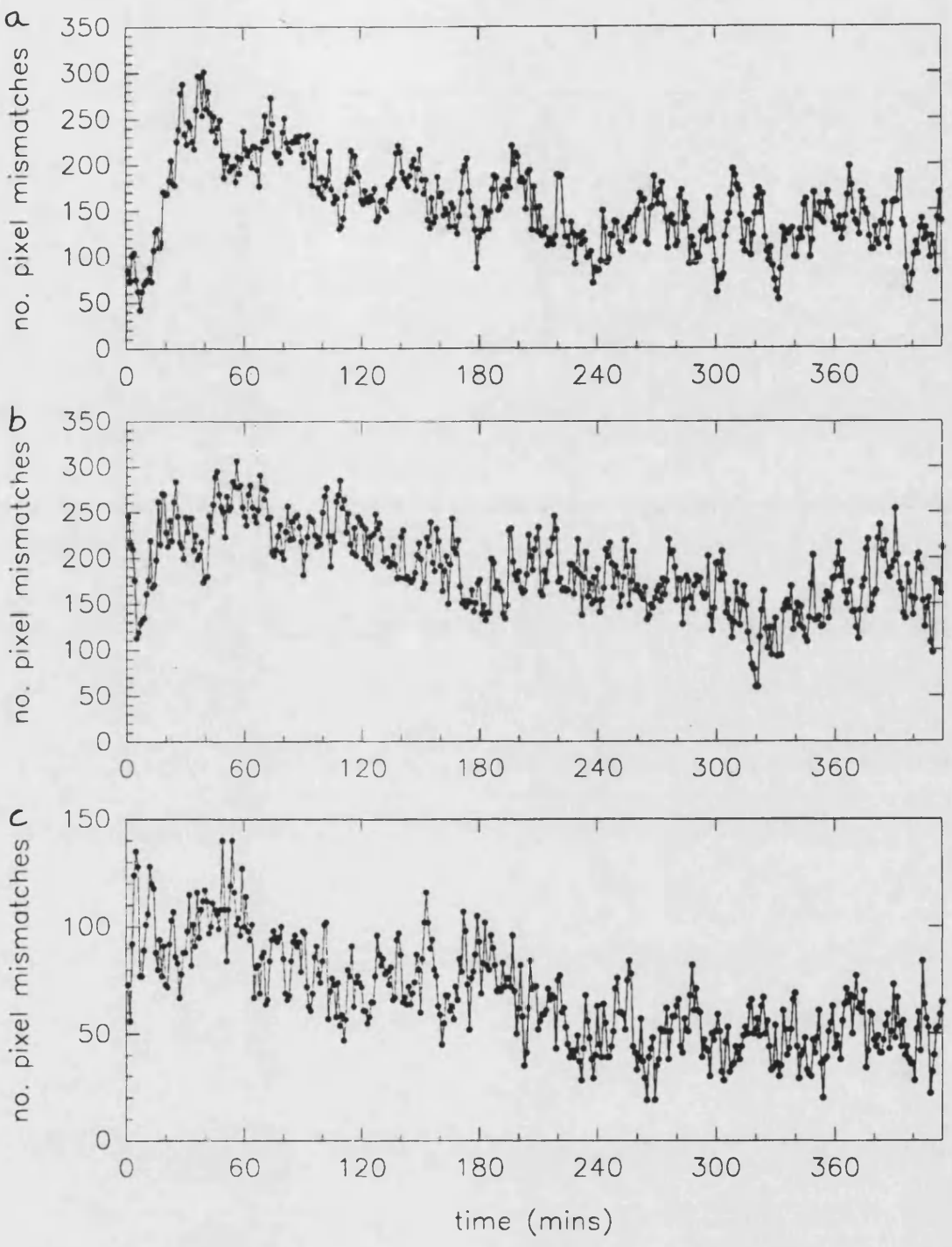

Figure 6.7: Activity time series from various windows on the day of food return: STA. a, window 2; b, window X1; c, window X2.

section:

- Response to food return is fast: food is discovered and reported to the nest within 5 minutes of its replacement; overall activity peaks within 1 hour.
- At no point do all individuals switch tasks to foraging: no more than 30% are observed to engage in it at any time, the nest contains no less than 70% of adults.
- Within the nest, food is initially exchanged away from the brood pile; there is an initial decrease in numbers of adults on the brood pile followed by an increase, suggesting that even under extreme stress food is passed via intermediate workers to the brood: replete foragers do not regurgitate directly to the brood.
- After activity on the brood pile has peaked, activity in other parts of the nest continues to rise for some time and food exchange between adults continues, suggesting that to some extent adults meet their personal energy requirments after those of the brood have been met.
- Activity cycles break down during the initial general peak in activity, but are restored within 3 hours of food return.

6.3.4 Activity level

Figure 6.8 depicts the evolution of daily mean activity level in STA. Contrary to Hemerik at al.'s (1990) predictions, there appears to be a downward trend in activity level as starvation proceeds, in the petri dish as a whole (Figure 6.8 a) and over the brood pile (Figure 6.8 b). Students T test was employed to assess differences in mean activity level for batches of days (as given in Table

6.3.2). In the case of STA, there is a significant decrease ($P< 0.05$) in activity level over the whole petri dish, brood pile, window X1 and also outside the nest as starvation continues (Figure 6.9 a-d). The nest 2ST appears to behave differently: there is no significant decrease in overall activity although the trend is downwards (Figure 6.9 e). Activity decreases significantly on the brood pile in the latter stage of starvation, and also after food is returned (Figure 6.9 f; see also Appendix D.6). There is no obvious relationship between activity in window X1 and food deprivation, but there is a significant increase in activity outside the nest under starvation (Figure 6.9 g, h). For both runs, there does not generally appear to be a recovery in activity levels to those prior to starvation after food is returned. This feature may suggest cautious interpretation of the data with reference to starvation. However such trends were not observed in other experiments in which food was available *ad libitum* (Chapters 4 and 7).

Correlation coefficients for activity levels in various parts of the nest also indicate a possible increase in cohesion between various groups of ants. Table 6.3.8 presents the score of significant correlations (Pearson product moment; $P< 0.05$) between windows representing regions within and outside the nest for batches of days (see also Chapter 4). In common with the results presented in Section 4.4.2, and contrary to the assumptions of Hemerik et al. (1990), for periods when food is available there is generally little correlation between foraging activity (as measured by activity at the food and water tubes, entrance, or total activity outside the nest) and activity within the nest, especially at the brood pile. However, as deprivation is continued there appears to be an increase in correlations of activity levels inside and outside the nest; to the extent that brood pile activity correlates on occassions with activity at the entrance, and even with activity at the food and water tubes. These correlations tend to reduce after food is returned, although correlations between activity in the nest and that at the entrance remain high,

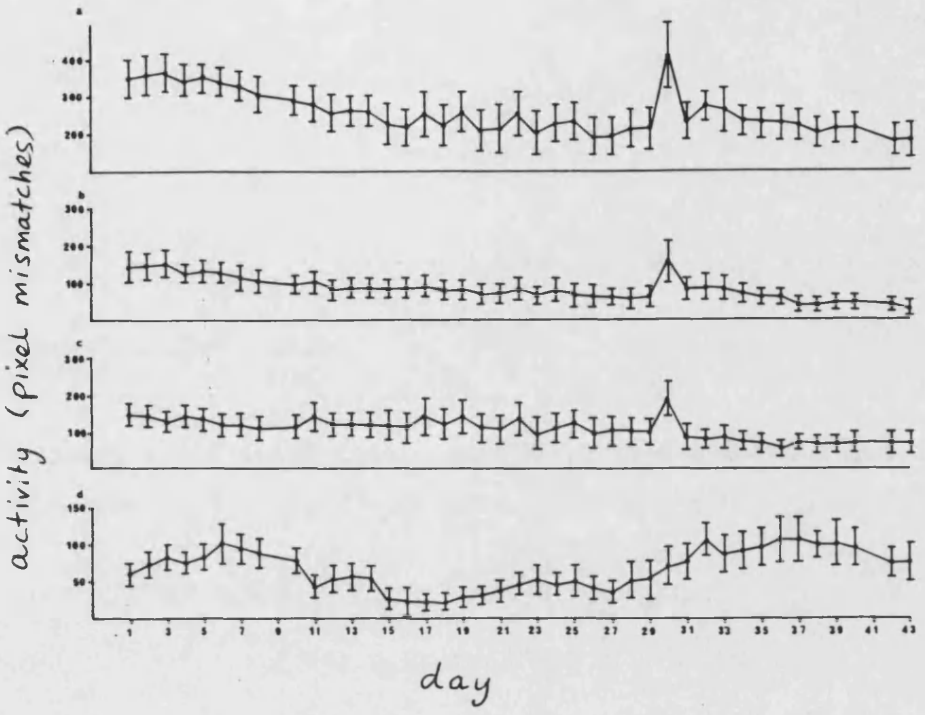

Figure 6.8: Mean and standard deviation of daily activity level (No. of pixel changes) in various windows for the run STA. a, window X0; b, window 2; c, window X1; d, window X2.

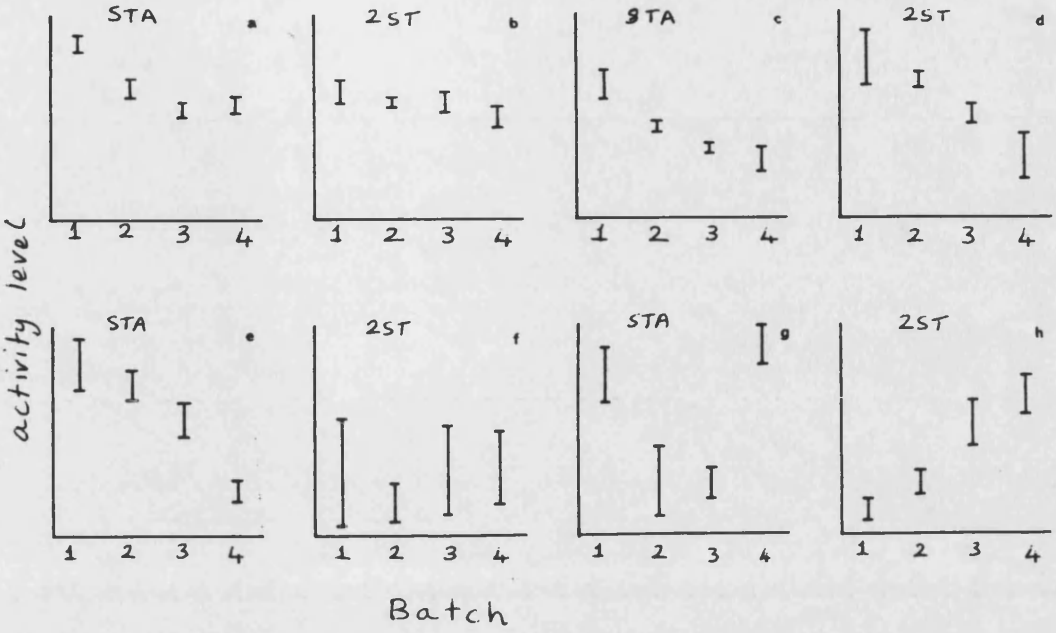

Figure 6.9: Relationship between activity level in various windows under starvation. The 95% confidence interval (from Students T test) on activity level is plotted for each batch. Batches 1 and 4 concern days when food was present; batches 2 and 3 days when the nest was deprived of food. a,b, window X0; c,d, window 2; e,f, window X1; g,h, window X2.

suggesting that some aspects of behavioural organization do not recover quickly following food deprivation, as noted above for mean activity level.

Table 6.3.8

		1	2	X1
STA(1)	*X2*	*4*	*2*	*5*
(max 8)	*X3*	*2*	*-1*	*3*
	5+6	*-1*	*1*	*-1*

		1	2	X1
STA(2)	*X2*	*5*	*0*	*6*
(max 10)	*X3*	*10*	*1*	*10*
	5+6	*2*	*1*	*0*

		1	2	X1
STA(3)	*X2*	*9*	*1*	*9*
(max 10)	*X3*	*10*	*3*	*10*
	5+6	*7*	*3*	*7*

STA(4)
(max 12)

	1	2	X1
X2	4	0	6
X3	12	4	12
5+6	1	2	2

2STA(1)
(max 10)

	1	2	X1
X2	1	1	6
X3	10	2	10
5+6	-1	-2	0

2STA(2)
(max 10)

	1	2	X1
X2	2	2	4
X3	9	7	10
5+6	0	0	1

2STA(3)
(max 10)

	1	2	X1
X2	5	2	9
X3	9	8	10
5+6	2	1	2

2STA(4)
(max 10)

	1	2	X1
X2	5	1	7
X3	10	6	10
5+6	0	0	2

Correlations between activity in various windows. Scores indicate the number of significant correlations ($P< 0.05$) between activity in pairs of windows (as marked). The maximum possible score is indicated in parentheses following the batch name for each table.

In summary, as food deprivation is continued, there is an overall decrease in activity which is especially marked on the brood pile. Activity that might be associated with foraging (activity outside the nest and within the nest near the entrance) tends to increase in the case of 2ST, but decreases in STA, and levels of activity in different portions of the nest tend to correlate more strongly in both experiments.

These features have plausible explanations by adaptive reasoning. Firstly, al-

though net energy expenditure may be reduced (less overall activity), the portion of energy allocated to searching for food is increased (higher entrance and outside activity relative to brood work). Also, whilst brood workers are less active, their level of activity tends to reflect more closely that at the entrance (increased correlations between portions of the nest); this may indicate that brood workers become more responsive to potential foragers, enabling a faster colony response to the return of food, should it occur.

6.3.5 Video analysis of individuals

The data from analysis of individuals from video recordings of the nest agree in general with the conclusions drawn from digitized data. Log survivorship plots of duration of inactivity bouts are roughly of the form expected from the WSCCS model: a near horizontal shoulder followed by a linear decay (see also Section 4.4.3). This suggests that individuals are unlikely to become active before a certain roughly fixed time after the onset of sleep, and waking after this lag time is probabilistic leading to a geometric distribution in sleep and cycle time (Figure 6.10).

In common with data presented in Chapter 5, the occurrence of spontaneous waking is rare in both task groups, representing 9.6% of events for fed ants and dropping to 2.7% of events for starved ants (Table 6.3.9). Amalgamating data across task groups, the number of spontaneous wakings is significantly lower ($P < 0.05$) for starved ants than for fed ants (Table 6.3.10).

Table 6.3.9

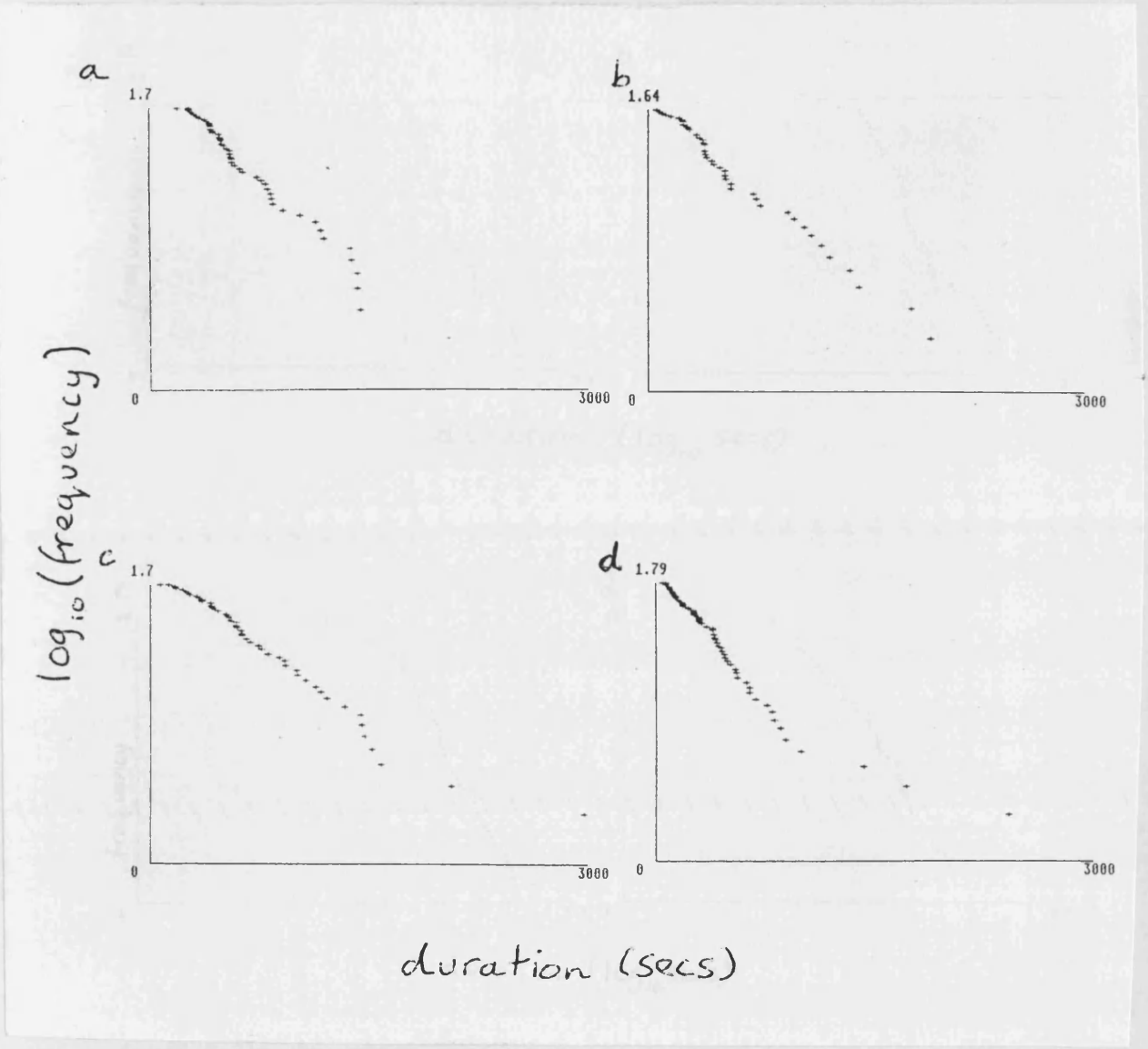

Figure 6.10: Log survivorship plots of inactive phase duration of individuals: 2ST. 2SF, after food return; 2SS, during food deprivation. Each point shows the number of events that were longer than the duration shown on the horizontal axis. BW, brood workers; DH, door hangers. a, 2SFBW; b, 2SFDH; c, 2SSBW; d, 2SSDH.

Run	W_s	W_c	*Totals*
2SFBW	*7*	*49*	*56*
2SFDH	*3*	*44*	*49*
2SSBW	*2*	*48*	*50*
2SSDH	*1*	*61*	*62*
Totals	*13*	*203*	*216*

Frequency of waking events that occurred spontaneously (W_s) or directly after contact (W_c). The data is presented for each task group (BW, brood workers; DH, door hangers) for ants after food return (2SF) and during starvation (2SS).

Table 6.3.10

	W_s	W_c	*Totals*
2SF	*10* *(6.26)*	*94* *(97.74)*	*104*
2SS	*3* *(6.74)*	*109* *(105.26)*	*112*
Totals	*13*	*203*	*216*

$X^2_{0.05,1df} = 4.58$

Association between spontaneous waking and starvation. For starved (2SS) and fed (2SF) ants, observed values of spontaneous waking (W_S) and waking after contact (W_c) are given; expected values in parentheses. χ^2 value (given above) compared to $X^2_{0.05,1df} = 3.8$ indicates significant negative association between W_s and starvation.

Table 6.3.11 presents the mean and variance of sleep and wake bout lengths and cycle times for brood workers and door hangers from the recording of 2ST during starved and fed periods. Significant differences (P<0.05, 95% confidence intervals; T test) between bout lengths are summarized in Figure 6.11.

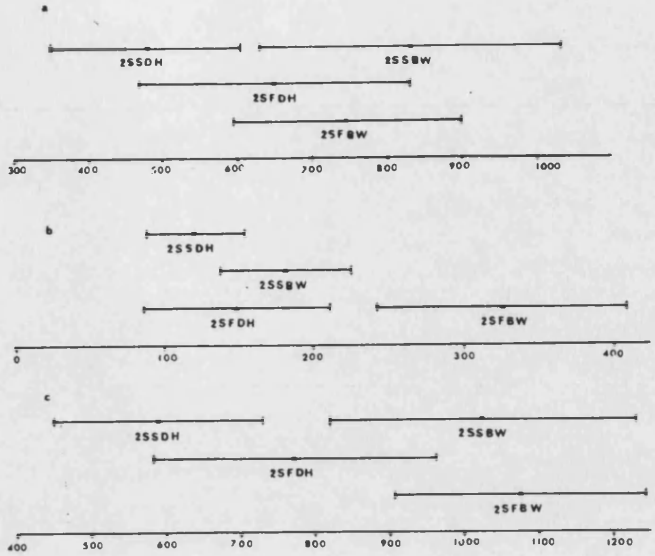

Figure 6.11: 95% confidence intervals of bout length (in seconds) for starved (2SS) and fed (2SF) individuals. Task groups: BW, brood workers; DH, door hangers. The confidence intervals were calculated from Student's T test. a, inactive phase duration; b, active phase duration; c, cycle duration.

Table 6.3.11

	Group	$n(x)$	$\overline{x}$	*Sd*
Sleep:	*2SFBW*	*50*	*745.96*	*534.6*
	2SFDH	*44*	*649.18*	*591.38*
	2SSBW	*50*	*831.00*	*697.25*
	2SSDH	*62*	*479.26*	*510.24*

	Group	$n(x)$	$\overline{x}$	*Sd*
Wake:	*2SFBW*	*55*	*326.27*	*299.56*
	2SFDH	*48*	*149.38*	*209.94*
	2SSBW	*55*	*181.80*	*155.83*
	2SSDH	*65*	*121.63*	*133.85*

	Group	$n(x)$	$\overline{x}$	*Sd*
WS:	*2SFBW*	*50*	*1074.76*	*582.88*
	2SFDH	*44*	*772.77*	*620.11*
	2SSBW	*50*	*1022.00*	*694.89*
	2SSDH	*62*	*591.86*	*551.32*

Summary of bout duration of brood workers(BW) and door hangers(DH) during starved (2SS) and fed (2SF) periods for the nest 2ST. Bout identity as marked for each table; $n(x)$, number of events; $\overline{x}$ the mean (in seconds); Sd standard deviation of x.

The data suggest that there are some differences in bout lengths between the two task groups generally (in agreement with the data presented in Chapter 5); door hangers have shorter active bouts than brood workers (significantly so for fed ants; $P<0.05$); and have shorter inactive bouts (significantly so for starved ants; $P<0.05$), leading to a shorter cycle time compared to brood workers (significantly so for starved ants).

The data also indicate some degree of response to food deprivation by the two task groups, the effect of which is in line with conclusions drawn from the digitized data for colony activity level. For brood workers, starving may lead to an increase in sleep duration (not significant) and a decrease in wake duration (significant); when considered together, there is little effect on cycle time. Such a behavioural shift, if it occurs, has a plausible adaptive explanation; energy resources are conserved by reducing the proportion of time spent active by individuals, thereby reducing the mean colony activity level, as suggested in Section 6.3.4 above. Door hangers appear to reduce both sleep and wake bout durations when deprived of food, leading to a decrease in cycle time, although not significantly so. These observations are thus in broad agreement with the digitized data for colony activity cycle length (a non significant decrease under starvation, and colony mean activity level (significant decrease under starvation).

6.4 Discussion

There is no evidence to support the main prediction of Hemerik et al. (1990) that colony activity cyles should break down after prolonged starvation. Visual inspection, autocorrelation and return maps of daily time series of pixel mismatches suggest neither a gradual reduction in cyclicity as starvation proceeds, nor a sudden breakdown in cyclicity. However, there is some evidence that cycle length may decrease slightly under starvation as suggested by Hemerik et al., (1990) although these differences are not significant ($P>0.05$). Increased sample size alone will not allow us to test for significance if the true decrease is only of the order of 1 minute, since the interframe interval (and thus the minimum experimental error) is itself +/- 1 minute.

From Figures 6.8 and 6.9, there is evidence that the general level of colony activity decreases as starvation proceeds, whereas the energy based model suggests that activity should increase. Taken naiively, the model's behaviour would seem counter intuitive, in that as energy availability decreases, the colony is predicted to expend resources at an increased rate. If the assumptions underlying the model were correct, this would be neccessary in order to allow increased foraging capability, since the number of foragers is assumed to be proportional to the total number of ants active in the colony. We see from the data however that the actual response of the colony may be more subtle: as starvation proceeds, activity on the brood pile appears to decrease whereas activity near the entrance and outside exhibits a variable response in the two colonies. Hence real colonies seem able to increase allocation of resources to foraging (i.e., searching for food), whilst allowing a net decrease in total energy expenditure (assuming expenditure per active adult is equal regardless of task). The data from observation of individuals support this view: brood workers show a significant decrease in the

duration of active phases. Also, brood workers tend to increase their sleep phase duration, whereas door hangers may decrease theirs. The subtlety of these behavioural shifts supports the notion that individuals do not behave identically, and provides further evidence for caste structure within the colony. Further, as starvation proceeds, there appears to be an increase in the proportion of active bouts (of individuals) that follow directly from physical contact. Hence, individuals rely more on stimulation from others, and are less likely to become active spontaneously. This feature also makes adaptive sense, in that the energy expenditure of individuals becomes weighted towards response to others, some of which might be returning successful foragers.

This observation is suppported by correlation results (Table 6.3.8) suggesting an increased coupling of activity between different parts of the nest under starvation. The activity level on the brood pile tends to correlate more strongly with that at the entrance and outside the nest as food deprivation continues. This may account for the observation from the digitized data that activity cycle length may decrease slightly, as it is governed more strongly by the (shorter) cycles of ants near the entrance.

Thus we do not observe the behaviour expected from Hemerik et al.'s (1990) model, when increased frequency of activity cycles is linked to increased colony activity, such that at higher activity levels cyclicity disappears altogether. Instead, we observe a slight increase in cycle frequency as groups of ants become more strongly coupled to the behaviour of the group with a shorter cycle length, although overall activity level drops. The observations of individuals, suggesting that brood workers increase their cycle length, need not be at odds with the observation at the colony level of decreased cycle length. Not all individuals neccessarily synchronize their activity to that of individuals at the entrance at

every cycle. So it is possible to observe individual brood workers with increased cycle length whilst observing shorter cycles for the whole colony, peaks in colony activity occuring when relatively more brood workers are active at the same time as doorhangers.

In summary, as starvation proceeds, cyclicity is maintained and activity appears to be more synchronized between groups, although it may be less synchronized within the brood pile itself. Individuals within the brood pile shift their allegiance (in terms of activity triggering) from each other to ants at the entrance. This shift may result in advantageous consequences for the colony, as the behaviour of ants near the entrance (potential foragers) becomes more influential on the colony as a whole. In effect, ants on the brood pile sample information more frequently from would be foragers when energy requirments are greatest; the frequent update of information may allow faster response when information concerning discovery of food reaches the nest. The increased linkage of brood workers to the behaviour of foragers may have a parallel explanation to that developed by Seeley (1989a) concerning Honeybee foraging dynamics (see also Section 8.3.1).

The data (Figures 6.6 a, b) suggest that within 2 hours of food return, most of the colonies' immediate energy deficits from 22 or more days of starvation have been met: activity at the sugar tubes and within all parts of the nest has peaked. During this response period activity cycles do indeed break down, as the colony maintains an increasing and then constant high activity level. Although the immediate response to food return is fast in terms of recovery of lower levels of activity and activity cycling, the data also suggest that food deprivation produces long term changes in the organization of activity, from which colonies do not appear to recover (at least within 10-12 days after food is returned). These changes include a continued overall reduction in activity, reduced activity on the

brood pile and increased coupling between brood workers and foragers. These changes may reflect changes in colony composition, for instance brood to worker ratio, perhaps induced by food stress. Alternatively, they may reflect responses on the part of workers to percieved changes in colony environment, such as an increased allocation to foraging in a habitat with reduced or variable reward rate. Another feature of interest is the apparent difference in response of the two nests to starvation; in one activity in window X1 and outside the nest decreases, whilst in the other activity in X1 remains stable but activity outside increases. These differences may reflect different colony compositions or colony age, as suggested by Gordon (1987, 1988 b).

In conclusion, these experiments suggest that the energy linked view of Hemerik et al. (1990) does not account for activity cycles within colonies. The prediction of cycle breakdown is not upheld, and the assumption of a linear relationship between foraging and colony activity levels does not appear to be met. We do observe a slight trend in decreasing cycle length, as predicted, but the mechanism proposed in the model to account for this differs from the account suggested by my interpretation of the data. The observed robustness of cyclicity to the stress of food deprivation lends credence to the hypothesis that cyclical colony activity results from simple proximate causes such as autocatalytic waking, as suggested by Tofts (1990a) and Goss and Deneubourg (1988). However, the observed differences in response to starvation by individuals again indicates that a precise match between observed behaviour and that predicted by the simple WSCCS model (Tofts, 1990a) is unlikely, since the assumption that individuals are identical is not met.

Chapter 7

Activity Patterns And Spatial Structure Of Nests

7.1 Introduction

7.1.1 Signal Propagation

In Chapters 4 and 6, I concluded that in general, the model of Tofts (1990a) can account for the observed distribution of activity cycle lengths in *L. acervorum*. However, it was also clear that the precise distributions observed were not exact fits to a geometric decay after a fixed lag; rather they appear to be the result of convolution between a normal and a geometric distribution (Figures 4.7). In Chapter 5, I investigated one possible refinement of the model: that of individuals being nonidentical with respect to their activity patterns. Another possible source of error in the model is the assumption that signal propagation is fast compared to the period of a completed cycle. Clearly then, it would be instructive to obtain an estimate of signal propagation speed.

For simplicity, the model assumes that signal propagation is a unitary process: an individual on waking is assumed to transmit to all others that can receive the signal (those that are 'wakeable') in a single step. In reality, this process must consist of a number of substeps. If the signal is transmitted as a chain of physical contacts between individuals, a given sender is unlikely to transmit successfully to more than a few individuals in any step. The number of steps required to transmit the signal across the whole nest and the duration of each step will dictate the speed with which the signal reaches the whole nest. If individual signal steps are long, or a large number are required, the overall wake up signal might be expected not to conform to the assumption of Tofts (1990a).

In the argument below I assume that an individual cannot send a signal until it is active, and that individuals become active on receipt of the signal. A signal step is the time neccessary for one such interaction between sender and receiver to occur.

Let us consider a brood pile containing N workers arranged as a single cluster. For simplicity, I shall assume it to be square; hence the group is arranged as a block of $N^{0.5} \times N^{0.5}$ individuals (Figure 7.1a). Once active, an individual transmits a 'wake up' signal to all its direct neighbours, which in turn become active if previously inactive. With this arrangment, if a single individual becomes active, no more than $(N^{0.5}) - 1$ steps are required for the whole nest to become active. Hence in Figure 7.1a, where 100 ants are arranged as a 10×10 block, in the worst case that a corner individual becomes active first, only 9 signal steps will transmit the signal across the whole group.

If however, the ants are arranged linearly as a $1 \times N$ array (Figure 7.1 c), between $N - 1$ (worst case: an end individual wakes first) and $(\frac{N}{2}) - 1$ (best case: the

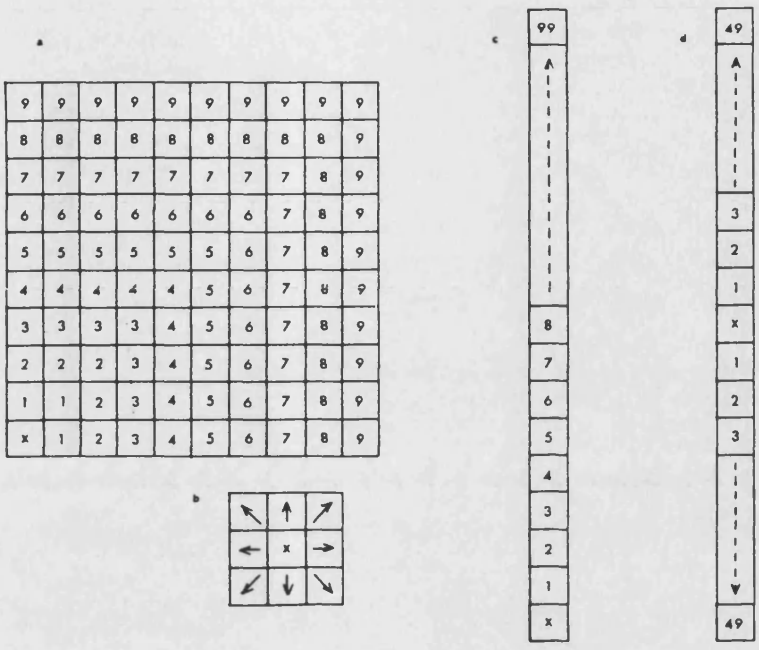

Figure 7.1: Hypothetical arrangement of ants: a, 10 by 10 cluster. If first ant to wake is X, the minimal number of steps to complete waking is 9 as indicated by the step number in each cell. b, assumed contacts, X may contact all direct neighbours as shown. c, a linear array 1 by 100: if first ant to wake is X, the signal must pass via all 99 to complete waking. d, best case scenario for the linear array.

middle ant wakes first; Figure 7.1 d) signal steps are required for activity to spread across the whole group. Hence, the linear arrangement will result in a total transmission time (for activation of the whole colony from a single starting individual) which exceeds that for the square arrangement by a factor of $N^{0.5}$.

In effect, the positive feedback element hypothesized to account for fast wake up and synchronization (Goss and Deneubourg, 1988; Tofts, 1990a) will not occur in a linear arrangement. The number that can become active at any step is now independent of the number already active, since activity onset is mediated only at two (or one) wave fronts containing one ant each.

This hypothetical discussion illustrates that in laboratory colonies consis ting of a single roughly circular brood cluster, the time neccessary to induce activity of the whole cluster might be expected to be very short relative to cycle duration. This is so since the required number of steps of signal propagation is low relative to the number of individuals present; interindividual connectivity is high owing to their spatial arrangement.

This argument holds provided that the duration of each signal step is short. From observation of individuals (Chapter 5), individuals become active within 1 second of being contacted by the 'sender'. Even if signal steps are of the order of 10 seconds (including time taken for a sender to move into proximity with a receiver), we can expect most individuals in a group of 100 to become active within 90 seconds of the onset of activity. This interval is short compared to the overall cycle duration of 20 minutes and comparable to the minimum experimental error (+/-1 minute) resulting from sampling positional change at 1 minute intervals.

If we wish to measure the signal propagation rate, it would seem reasonable to confine the signal spread to one dimension, and attempt to measure its propagation in colonies that have been forced into a linear arrangement. We might also expect colony level cycles to break down in a linear arrangement, as a result of two factors:

- individuals no longer synchronize their wake and sleep bouts, as the time to propagate the signal across the whole nest exceeds the wake bout duration of individuals. Hence individuals that wake early have already become inactive by the time the 'wake up' signal reaches individuals at the other end of the chain;
- there is a higher probability that the wake up signal will fail to propagate across the whole nest. If the signal is transmitted by direct (physical) propagation between each individual and the next, if one element in the chain fails to respond, the signal will not be passed on to all elements distal to the failed connection in the series. If the ants are arranged as a cluster, a small increase in individual transmission steps can circumvent such 'failed' elements (see Figure 7.2).

Preliminary Experiment

I attempted to investigate these predictions by measuring the activity levels in a colony forced to inhabit a nest essentially linear in structure. Activity levels were measured using the automated image analysis techniques described in Chapters 3,4 and 6. By taking frames at 10 second intervals (the minimum interframe period possible with this equipment: Chapter 3), I hoped also to measure the speed of propagation of the wake up signal.

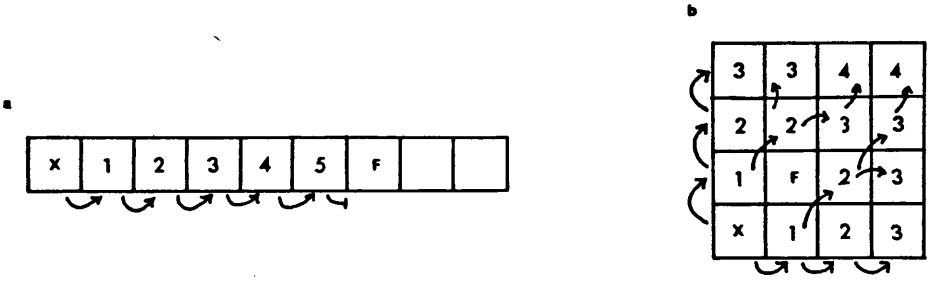

Figure 7.2: The effects of 'failed' transmission elements in signal propagation. (a) linear arrangement. All elements to the right of F are not reached by the signal. (b) Square arrangement. Arrows indicate on possible route of transmission around a failed element F.

I constructed nests that allowed only a single ant to travel and turn around inside the chamber, by cutting a channel of appropriate dimensions into card and sandwiching this between glass slides. Colonies were encouraged to move into these nests by splitting apart the old nest and placing the broken nest and contents into a plastic arena together with the new nest. However, I found that colonies would not move into nests with such a narrow chamber, even after several days exposure in the arena. Colonies would however move into slightly wider chambers in which 2 ants could rest or move side by side. I constructed a nest with a linear chamber 400 $\times$ 2 $\times$ 3 mm in dimensions by cutting an appropriate channel into cardboard (see Figure 7.3), and sandwiching between glass slides. A colony containing 64 adults was allowed to immigrate by the above method.

Using the automated techniques described in Chapter 4, I measured the activity level at ten second intervals in each of forty windows covering consecutive portions of the nest, and on another occasion at one minute intervals.

The time series for overall activity (one minute intervals) did not appear to differ markedly to that of other nests on inspection (see Appendix D.1); it still appeared cyclical in form, and did not oscillate in a random fashion (Turning point

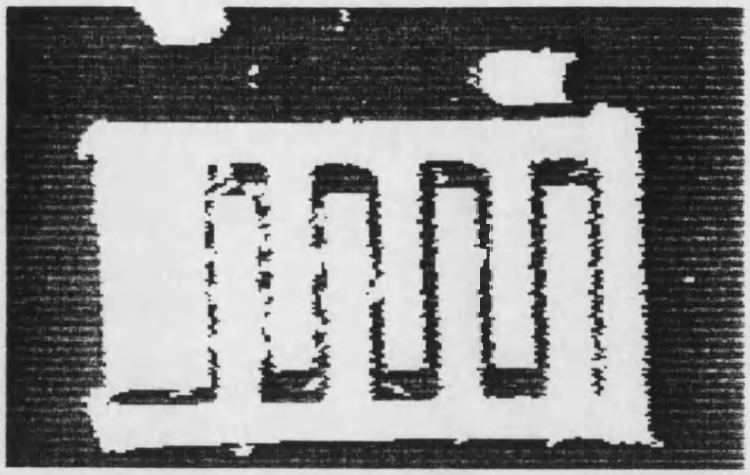

Figure 7.3: Digitized picture of a 'linear' ant nest. Ants and brood (white) are shown against the black background of the chamber, the shape of which is constrained by the cardboard nest wall (in white).

statistics, Table B.0.8).

Signal propagation was investigated by searching the time series at 10 second intervals of individual windows for consecutive activity peaks (in time) in consecutive windows (neighbouring in space). I hoped to see an initial burst of activity in a focal window transmitted in the following time intervals to nearest neighbours on one or both sides of the focal window. Although regions in the timeseries did illustrate this phenomenon for regions of the nest, the overall appearance of the data suggested that propagation was still too fast to be measured from data taken at 10 second intervals. For instance, activity peaks frequently appeared to 'jump' across windows or occur in different windows at the same time . The former condition would arise if some windows did not contain individuals when the frames were snatched, such that individuals ran through a window during the interframe interval, but their movement was not recorded. It would also occur if individuals within the window remained stationary but others were able to pass. Simultaneous peak activity in different windows might arise if signal propagation between windows took less than 10 seconds.

However, the pattern of correlations between activity levels (10 second intervals) in different windows suggested that activity within different regions were poorly coupled to each other (see Appendix D.7 for sample); significant positive correlations between neighbouring windows were common, but were infrequent between more distant regions of the nest.

The correlation results must be interpreted with caution, since activity levels in individual windows were low. Also, correlations between direct neighbours might result from single ants that occupied positions across the borders of windows.

Although the concept was of heuristic value, the experiment itself yielded little information. Firstly, it is difficult to measure the speed of propagation directly, as it is probably too fast to be clear from comparison of images at 10 second intervals. If the interframe interval is reduced sufficiently, another problem arises; the actual amount of change between images becomes so small that noise filtering techniques may also remove any signal, and the length of time series that can be collected is so short that we are unlikely to record any events of interest. Secondly, indirect techniques such as inspection of time series and patterns of correlation between the different regions are also problematic. Since Fourier analysis and autocorrelation are inappropriate, we have no direct method for ascertaining statistically whether cycles overall exists; and since activity levels within windows are extremely low, it is difficult to interpret patterns of correlation which may result entirely from the activity patterns of a few individuals.

7.2 Nest Structure And Oscillator Coupling

7.2.1 Introduction

Naturally occuring nests of *L. acervorum* tend to consist of a ramifying network of interconnecting chambers within branches or bark (Collingwood, 1979). Hence the single chambered laboratory nests considered so far are a simplification of naturally occuring nests, although they can occur (for example, nests in rock crevices may contain only a single chamber; N.R. Franks, pers. comm.), and must initially be arranged as such in incipient colonies. I present evidence in Chapter 4 that the frequency of cycles of colony activity is independent of colony size, for colonies larger than *circa* 12 individuals. However, from the models of Tofts (1990a) and Goss and Deneubourg (1988), cycles may not occur or their frequency may be much reduced for smaller colonies. Hence it may be possible to cause a breakdown in overall colony activity cycles by manipulating the physical structure of the nest such that it consists of small interconnecting chambers. Unless interactions between chambers are fast and frequent, we might not expect to observe cyclicity over the whole colony. Also, if individual chambers contain few ants, cyclical activity patterns may not be found within chambers, or might occur at reduced frequency (Tofts 1990a). This latter expectation results from a reduced likelihood of activity triggering at any instant. For a colony activity peak to occur after complete inactivity, at least one individual must wake spontaneously and stimulate others to activity. The frequency of spontaneous wakings, and hence of cycles in a perfectly synchronized group, will be dependent upon the number of individuals in that group.

Hence, in a nest structure more reminiscent of naturally occuring forms, we might

expect:

- no overall colony activity cycles, if strength of interactions between chambers is low;
- cycles of activity within chambers, but at a lower frequency than those found in single chamber large nests. Alternatively, cycles may not occur at all if the frequency of spontaneous wakings of individuals is too low.

Physical structure and colony organization

In Chapter 4 I concluded that the activity of brood workers was not strongly linked to that of foragers, as significant correlations between activity levels in windows representing these groups rarely occured. In Chapter 6, I presented evidence that this relationship changed under the stress of starvation: the activity level within the brood pile became more closely correlated to that at the entrance and outside the nest. In Section 7.1, correlations of activity levels within windows revealed some link between activity level and physical proximity, since activity levels were more frequently correlated between neighbouring regions than to distant regions.

Hence, by measuring the correlation of activity levels between various portions of the nest, we may be able to gain some insight into interactions between subsections of the colony. Correlation between activity levels might thus be used as a tool for investigating colony behavioural and structural organization, independent of the precise patterns or levels of activity (provided they are nonzero). We must, however, first consider a number of conceptual issues.

1. **Covariance and causality.** Correlation coefficients are a measure of the covariance in a pair of variates A and B; these being simultaneous time series of activity levels within various portions of the nest. As such, we conclude from significant correlations ($P<0.05$) that 95% of such correlations result from nonrandom covariation between A and B. We cannot attribute causality in this conclusion; with this data alone it is not possible to state that variation in A contributes any given proportion of the signal in B, or *vice versa.* For instance, if all pairs of windows were shown to correlate in activity level, we might suspect an external cause such as environmental factors, a global clock, or possession of an accurate clock by all individuals acting independently.

2. **Significance and cyclicity.** For a significance judgement of $P<0.05$, we can conclude for noncyclical variates that less than 1 in 20 such correlations result from chance covariance. However, it is not clear that such is the case for cyclical variates (Figure 7.4). Consider two independent variables A and B, which cycle at equal frequencies. If the onset of a cycle (X and X_1: Figure 7.4) happens to coincide for both variables, strong correlation will be reported between the two. The onset of a cycle in B within some range 0+/- T/4 of the onset in A's cycle (where T is the period of both cycles) will lead to a positive correlation coefficient some of which will be significant. Onset of B within 1/2 +/- T/4 similarly leads to a negative coefficient. For cycles of variable length and asymmetric shape, the relationship between chance coincidence of independent cycles and reinforcement of the coefficient of correlation is less clear. Hence it is neccessary to determine empirically what constitutes a correlation that is unlikely to be the result of chance covariance (see Section 7.2.3).

3. **Pixel mismatches and correlation.** When the variates under consider-

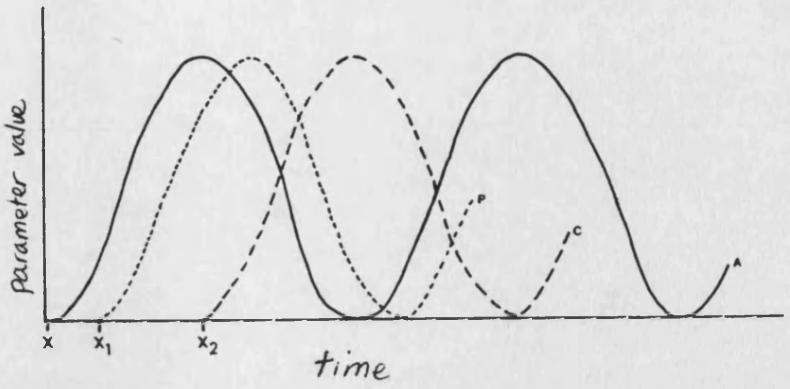

Figure 7.4: Chance superposition of cycles: onset of cycle B (dotted) at X_1 results in a positive correlation between A and B. Onset of cycle C (dashed) at X_2 results is a strong negative correlation between A and C, where onset of A is at X.

ation are pixel mismatches in various portions of the nest, we can expect some degree of positive covariance in activity levels between direct neighbours. This occurs since any movement across a common border will register as an increase in the number of pixel mismatches in both windows (see Chapter 3). To the extent that movement across borders is large compared to movement within borders, we may overestimate correlations in activity level between physically connected windows. Hence if the only significant correlations occured between direct neighbours, we might wish to conclude the correlations are artifacts of the activity of individuals that happen to lie across borders, rather than a true indication of connectivity in activity levels within windows.

4. **Correlation and automation.** One advantage of this technique is that it requires no human intervention in data processing. Since we are not concerned with cycle duration, no human judgement on phase reference points is necessary. Each value of the raw data (number of pixel mismatches) collected automatically contributes to the calculation.

With these considerations in mind, I conducted a number of experiments to assess

1. whether cycles of activity occur overall and within chambers of a multi-chambered nest;

2. whether correlations between activity levels in different windows yield insight into colony organization in multichambered and single chambered nests.

7.2.2 Methods

Multichamber design

A nest consisting of 8 chambers connected by a central passage was designed by making appropriate cuts in a 60 × 40 × 2 mm piece of card. The card was then sandwiched between two 60 × 40 × 1 mm glass slides and sealed with Scotch sellotape to produce an 8 chambered nest with a single entrance (Figure 7.5). A colony of *L. acervorum* containing 149 adults (Table 7.2.1) was encouraged to move into the nest by breaking the old nest and placing it together with the new in a plastic arena. The colony was allowed to acclimatize to the new nest in standard laboratory conditions (Chapter 2) for 3 weeks before experiments commenced.

Table 7.2.1

Colony	*W*	*Q*	*E*	*S*	*Me*	*L*	*P*	*M*
1POK	*146*	*3*	*28*	*32*	*19*	*34*	*0*	*0*
GRID	*85*	*5*	*66*	*10*	*6*	*44*	*0*	*0*
BOX	*69*	*3*	*8*	*48*	*14*	*11*	*0*	*0*
TUBRUN	*135*	*1*	*23*	*54*	*27*	*15*	*0*	*0*

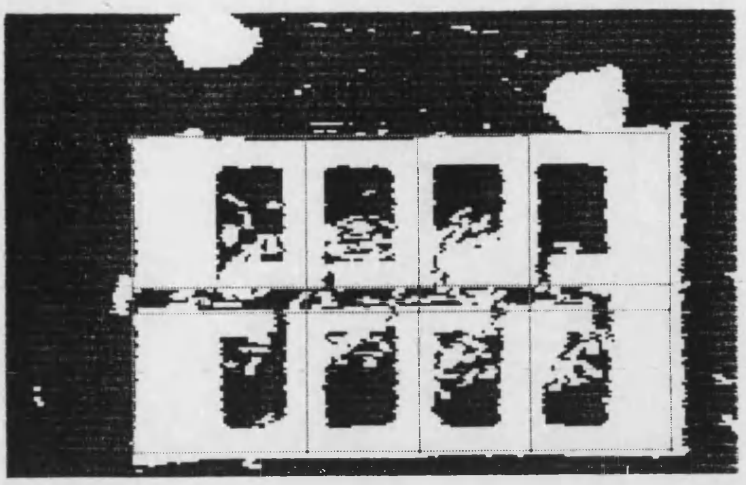

Figure 7.5: Digitized picture of multichambered nest (1POK). The nest consists of 8 chambers connected by a central passage to a single entrance. Ants and brood show as white objects against the black background of chambers.

Colony census data. For each run the number of items as characterized in Chapter 2 is given: W, workers; Q, Queens; E, eggs; S, Me, L small medium and large larvae; P, pupae; M, males.

Image analysis

For each of the four runs, the focal colony was placed in the constant temperature room 48 hours before filming commenced, hence allowing acclimatization to standard experimental conditions (i.e., lit from below, 25°C, 50% humidity). Image analysis proceeded as described in Chapter 4; commencing at 11 am, the whole petri dish was filmed, and 416 digitized frames were grabbed at 1 minute intervals. The images were analysed as described in Chapter 4 (and summarized in Table 7.2.2); pixel mismatches for consecutive frames were counted on the basis of false colouring decisions for 4 × 4 blocks of pixels, for the whole field of view and a number of windows representing various portions of the nest.

Table 7.2.2

Run	*Start Date*	*Days*	*Nest Design*	*W*
1POK	*29/8/91*	*16*	*8 Chamber*	*13*
GRID	*16/9/91*	*8*	*Single Chamber*	*13*
BOX	*26/9/91*	*10*	*Single Camber*	*13*
TUBRUN	*19/10/91*	*16*	*Single Chamber*	*13*

Summary of experiments. For each run, the start date indicates the first day of digitized data collection; Days indicates the number of days of image analysis. W, the number of windows within the nest for which activity levels were measured.

Four experimental runs were conducted; one for the multichambered nest (16 days); three control single chambered nests as utilized in Chapters 4 and 6, two of which involved colonies of *L. acervorum* (8 and 10 days each), and the other for *L. tubero-interruptus* (16 days; see Table 7.2.3).

Table 7.2.3

Number of Days	*8-16*
Frames per day	*416*
Frame interval	*1 minute*
Number of Windows	*15*
Block Size	*4 × 4*
Thresholds:	
lower	*8*
upper	*11*

Summary of image analysis for each run. See Chapter 4 for details.

For the mutichambered nest, partitions were set to cover each of the chambers (8) and sections of the central passage (4), plus entrance, making 13 windows in all

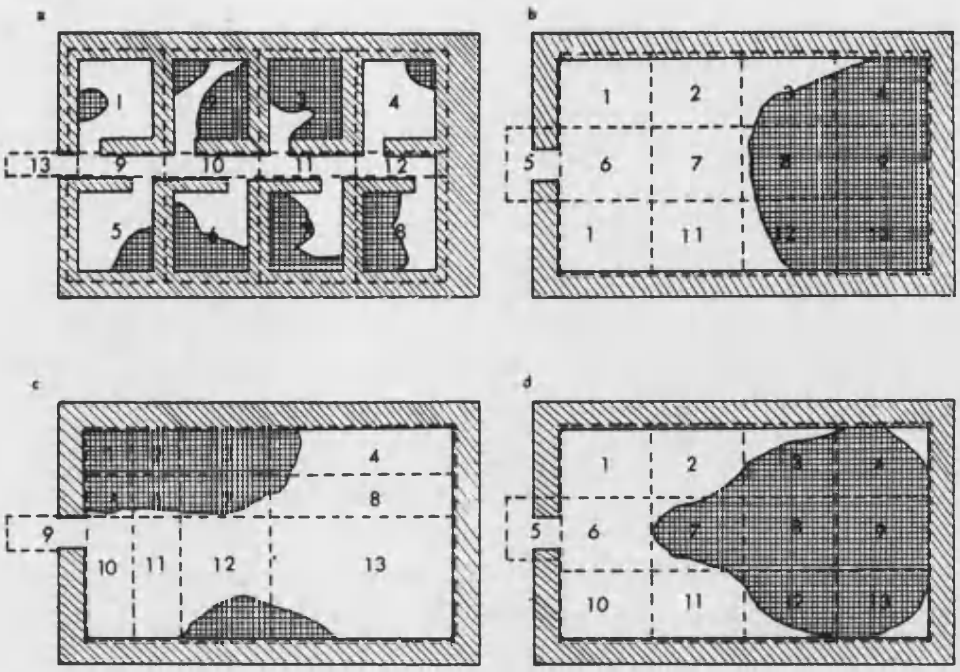

Figure 7.6: Nest design and partitions. Physical structure: chambers are delineated by the position of cardboard nest walls (diagonal hatching). Logical partitions: windows (as numbered) are formed by setting partitions (indicated by dotted lines). Position of the brood pile is shown by square hatched areas. a, 1POK; b, GRID; c, BOX; d, TUBRUN.

(plus whole nest and whole field of view). For each control nest, 12 windows were set to cover the nest, plus one over the entrance. In some cases these windows varied in size in relation to different densities of ants and brood within the nest (see Figure 7.6).

After completion of the daily run, the nest was inspected and food and water replaced if neccessary. Care was taken on such occassions when the position of the nest was disturbed to reset the positions of windows appropriately without changing their dimensions. The resulting timeseries of pixel mismatches for the whole nest and within windows were analysed as described below to investigate cyclicity and correlation of activity levels.

7.2.3 Results

Overall activity cycles

Figure 7.7 illustrates sample daily timeseries of activity for the whole nest for each run. By visual inspection, there is no evidence for a qualitative difference in timeseries for the multichambered nest (1POK): peaks and troughs in activity occur in each case (see also Appendix D.1). The activity timeseries do not appear to be random (Table 7.2.4: comparison of 95% confidence intervals of turning points to random expectation; see also Tables A.0.6 and B.0.9).

Table 7.2.4

Run Date	*N*	*TPs*	*LCI*	*UCI*
1POK01	*414*	*196*	*266*	*283*
GRID01	*413*	*208*	*265*	*283*
BOX01	*412*	*219*	*264*	*285*
TUBRUN01	*404*	*199*	*259*	*277*

Sample turning point statistics for the first day of each run, the number of independent data points (N) and number of observed turning points (TPs) is given. 95% confidence interval for the random expectation is shown by LCI and UCI.

Return maps and autocorrelation (see Appendices D.3 and D.4) also do not indicate a qualitative difference between the time series of overall activity in 1POK and single chambered nests.

Cycle length distribution was analysed as described in Chapter 4; activity troughs were located manually to obtain measurements of cycle length; daily measure-

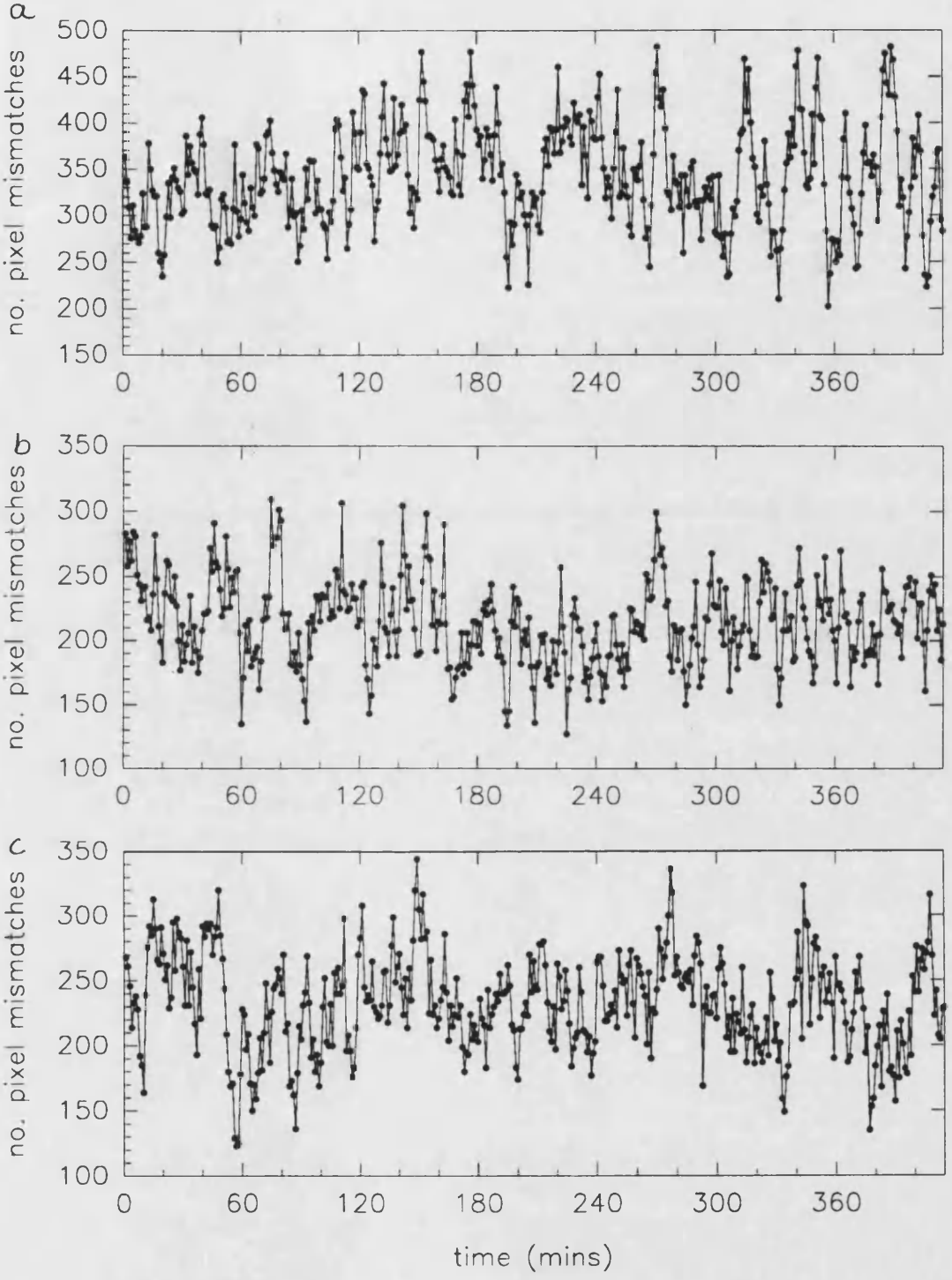

Figure 7.7: Sample activity time series (window X0). The day of each run is indicated following the run name: a, 1POK13; b, BOX07; c, GRID06.

ments were amalgamated to produce a single distribution for each run and compared to that expected from the WSCCS model (geometric decay after fixed lag) by χ^2 test for goodness of fit. The results (Table 7.2.5) indicate that the cycle distribution of the mutichambered nest does not fit any of nine predicted by the model (P<0.05) and that cycle distributions for the two single chambered nests involving *L. acervorum* are not significantly different (P>0.05; best fit distributions) to that expected. The distribution of cycle lengths in the case of TUBRUN, involving *L. tubero-interruptus*, has already been discussed in Chapter 4; it does not appear to fit any of nine expected distributions, and the mean interval between troughs is significantly longer (P< 0.05; T test) than that for *L. acervorum*. Mean cycle length for 1POK does not differ significantly from that in single chambered nests of *L. acervorum* (this Chapter, Chapters 4 and 6; P> 0.05, T test).

Table 7.2.5

Run	*N*	*Mean Length*	*MLE*	*Best* χ^2	*No.* χ^2 < 11.07	*No. Short cycles*
1POK	*275*	*1324*	*18.78*	*16.16*	*0*	*19*
GRID	*141*	*1280*	*8.08**	*5.81**	*4*	*8*
BOX	*174*	*1325*	*16.59*	*6.73**	*3*	*19*
TUBRUN	*218*	*1642*	*24.94*	*11.22*	*0*	*24*

Summary of goodness of fit to WSCCS model. Cycle length (in seconds) measured from window X0. N, number of cycles observed; Mean Length, mean cycle duration (in seconds); MLE, χ^2 goodness of fit (χ^2 value) to the maximum likelihood distribution; Best χ^2 goodness of fit to closest test distribution; No. $\chi^2 < 11.07$, the number of distributions (out of nine) that did not depart significantly from that of the data. The number of observed cycles shorter than the estimate for base sleep time is also shown.

The distribution of cycle lengths in 1POK does not appear to differ qualitatively from that in other nests (Figure 7.8 a; for others see Chapter 4 and Appendix D.5). Both the third and fourth moments depart significantly (P< 0.05) from the normal expectation (Table 7.2.6), indicating right skewness and leptokurtosis. Log survivorship and model estimates of minimum cycle length s also agree (Table 7.2.7) within the bounds of measurement error (+/- 1 minute).

This experiment would seem to require replication in order to form stronger conclusions as to whether activity patterns differ strongly from that in single chambered nests, or from that predicted by the WSCCS model.

Table 7.2.6

Run	*N*	*Mean*	*Variance*	g_1	g_2
1POK	*275*	*1324*	*201869*	*8.37**	*12.22**
GRID	*141*	*1280*	*179186*	*6.43**	*5.08**
BOX	*174*	*1325*	*170077*	*4.58**	*1.55*
TUBRUN	*218*	*1642*	*208247*	*3.29**	*0.31*

*Summary of cycle length statistics. Cycle length measured from window X0. N, Mean and Variance refer to the number of events, mean cycle length (in seconds) and variance. g_1, third moment; g_2, fourth moment. * indicates significant (P< 0.05) departure from normal expectation.*

Table 7.2.7

Run	s_l	s_g	$s_l - s_g$
1POK	896	874	22
GRID	885	856	29
BOX	867	912	-45
TUBRUN	1222	1185	37

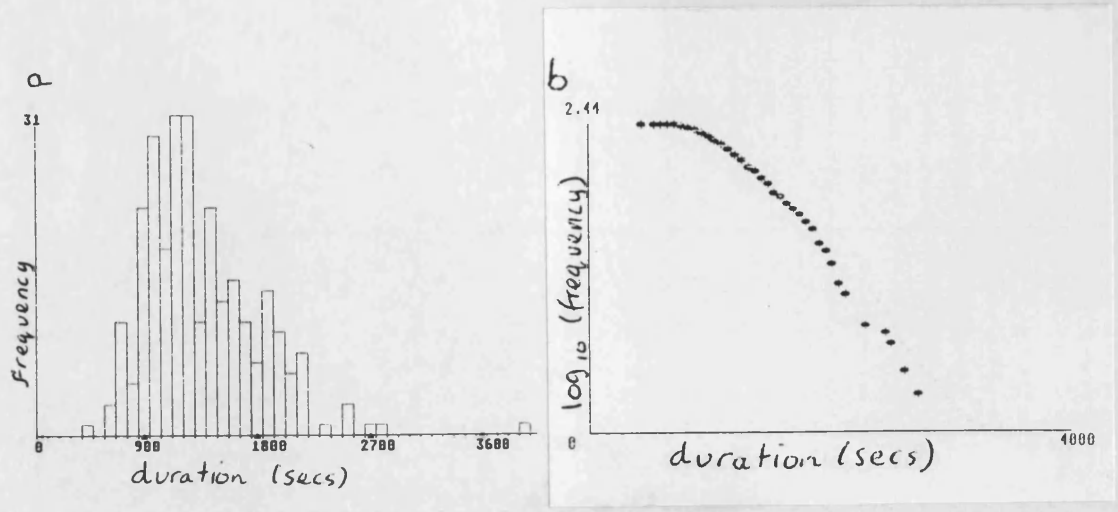

Figure 7.8: Distribution of cycle lengths (in seconds) in 1POK: a, frequency distribution; b, log survivorship function. b plots the number of cycles that were longer than the duration shown on the horizontal axis.

Comparison of base sleep time estimates (in seconds). s_l: log estimate of s based on intersection of lines fitted by inspection to log survivorship curves of colony cycle length. s_g: estimate of s from WSCCS model and data. Cycle lengths were measured from activity time series in window X0.

Cycles within pockets

Figure 7.9 presents sample daily timeseries for some chambers; visual inspection suggests that activity level tends to cycle; there are clear peaks and troughs in the time series. This is supported by analysis of turning points (Appendix B.0.10); the time series contain significantly fewer turning points ($P<0.05$) than expected for a random series. The amplitude in Figure 7.9 is neccessarily small compared to that for the whole nest, making cycle interpretation difficult: the timeseries are dominated by high frequency peaks at intervals in the order of 5-10 minutes. These peaks represent changes in amplitude of roughly 30 pixel mismatches, compared to peaks in whole colony data in the order of 100 mismatches. These high frequency low amplitude peaks may be the result of individuals entering and

leaving the chamber, rather than synchronized activity bouts within the chamber.

The time series data was processed using the moving minima procedure described in Chapter 4 to reveal troughs in activity that were stable at intervals longer than 8 minutes. Troughs were located manually, and the distribution of intertrough intervals compared to that expected from the WSCCS model (Table 7.2.8). There is evidence that in some chambers, longer term oscillations occur at roughly 35 minutes intervals, and are distributed in accordance to the WSCCS model ($P>0.05$; χ^2 goodness of fit).

Table 7.2.8

W	*N*	*Mean*	*Var*	*MLE* χ^2	*best* χ^2	*no.* χ^2 < 11.07	*short*	*Amp*
1	*174*	*2045*	*368185*	*31.7*	*19.6*	*0*	*26*	*17.7*
2	*175*	*2016*	*408977*	*37.7*	*7.6*	*3*	*22*	*18.4*
3	*161*	*2199*	*451906*	*17.04*	*8.06*	*3*	*16*	*19.1*
4	*170*	*2070*	*513120*	*6.21*	*3.99*	*2*	*18*	*21.7*
5	*162*	*2156*	*463796*	*26.1*	*11.7*	*0*	*19*	*26.5*
6	*163*	*2157*	*442914*	*12.11*	*9.8*	*1*	*21*	*22.2*
7	*172*	*2053*	*399864*	*10.7*	*6.5*	*2*	*16*	*23.2*
8	*160*	*2197*	*464560*	*13.9*	*9.5*	*1*	*19*	*26.5*

Cycle properties in the multichambered nest. For each window (W) set over a chamber, the number of cycles observed (N), mean cycle length (Mean; in seconds) and the variance is given. The distribution of cycle length was compared to nine test distributions; see Table 7.2.5 for explanation. Amp indicates the mean activity level (pixel mimatches) in each chamber.

The chambers in which activity patterns appear to fit the WSCCS prediction are generally those exhibiting higher mean activity levels, and are also situated

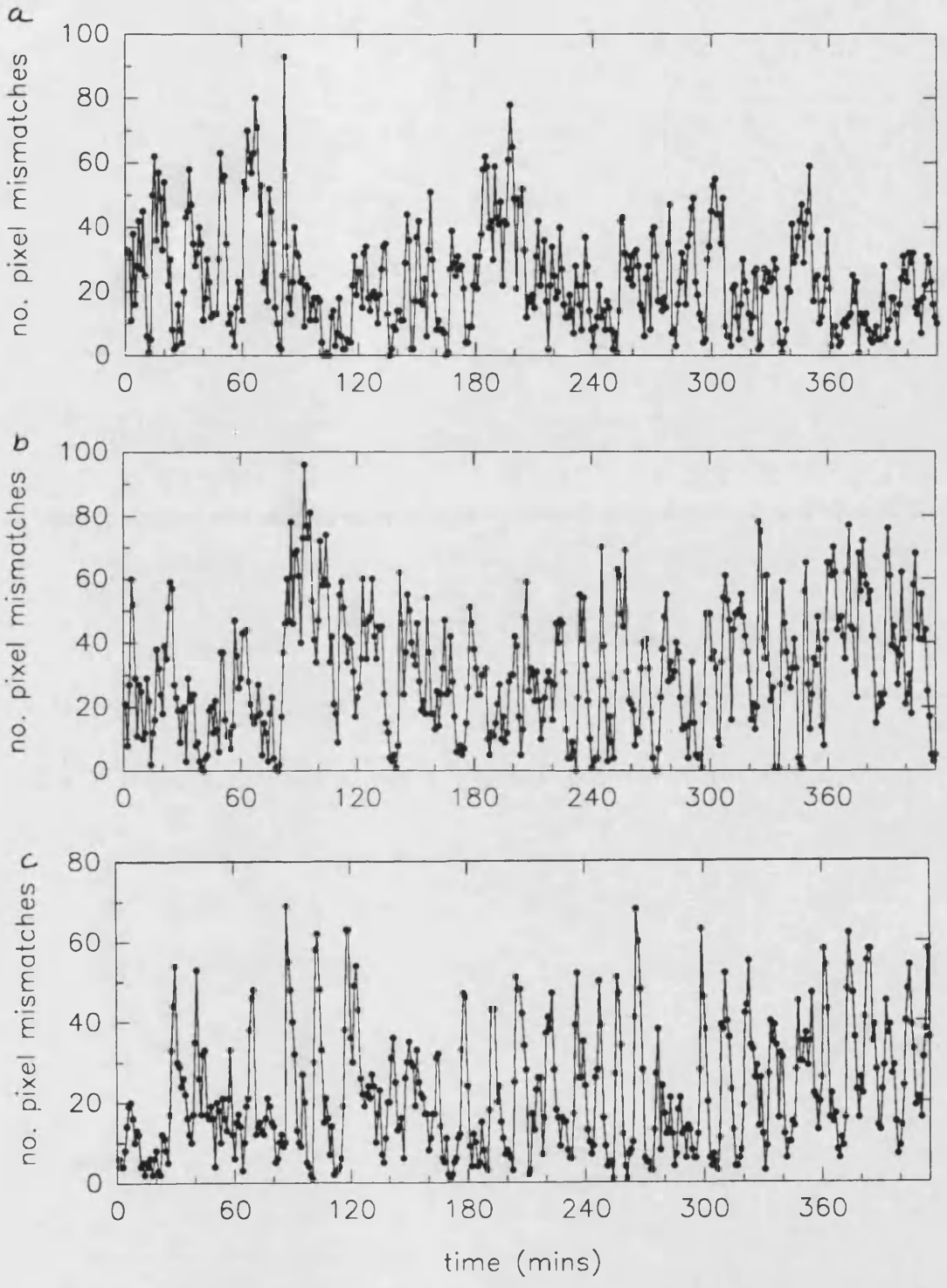

Figure 7.9: Sample activity time series for chambers in 1POK. Data is presented for day 07 of 1POK. a, chamber 5; b, chamber 6; c, chamber 7.

away from the nest entrance. It is possible that clearer cyclicity occurs in these chambers as a result of larger numbers of individuals in the chambers and less interference between brood workers and door hangers (see also Chapters 5 and 6). Again, analysis of cyclicity in individual chambers should be viewed with caution since the amplitude of activity change is low compared to that for the whole nest, and high frequency pulses of activity (which may be caused by very few ants) interfere with longer term oscillations. The apparent reduced frequency of cycles (35 minutes) in small chambers compared to that for larger nests (20 minutes) supports the prediction of Tofts (1990) that cycle length will increase when very few ants are involved (chambers contained between 9 and 15 ants).

Correlations: random estimate

In Section 7.2.1, I concluded that an estimate of the frequency of significant correlations (where $r_{obs} > r_{crit}$; $P<0.05$) that could be obtained as a result of random events was required. It is not possible to generate a mathematical formula for this, since the precise shape of the waves I shall correlate is unknown and compounded by stochastic elements. An estimate was made directly from the data, by choosing pairs of data files from different days of the same run at random, and performing cross correlations (as described in Appendix A.6).

Hence I obtained an estimate of the probability of obtaining a significant correlation between independent cyclical data sets, in that we expect the data generated on a given day to be independent of that generated on a different day. Each estimate is appropriate to the variates we wish to correlate, since it is derived from correlations between these variates (given pairs of windows, such as chambers in the nest of 1POK, or brood pile versus entrance in MIDRUN, 2ST etc; Chapters

4 and 6).

Correlations of activity level between regions of the nest

For each daily output, cross correlations were performed between activity levels in different windows within the nest. The windows analysed were the 8 chambers and 5 subsections of the central passage in 1POK (see Figure 7.6a), and the 13 partitions of the single chambered nests GRID, BOX and TUBRUN (Figure 7.6b-e). The number of significant correlations (where $r_{obs} > r_{crit,0.05}$) for each pairwise combination of windows (cells) were summed over each day (8-16 days for the 4 runs). The resulting single score in each cell for each run (Table B.0.11) was compared against 95% confidence intervals for the empirical estimate of random occurrence of significant correlations (Appendix A.6).

Observed scores lying outside the confidence intervals for the random estimate are taken to indicate positive correlation between activity levels in those windows; the information is summarized in Figure 7.10. No negative correlations were found to occur more frequently than expected by chance.

Firstly, in all cases activity within most logical or physical subsections of the nest is not correlated directly to activity at the entrance, in common with findings in Chapters 4 and 6. This is contrary to the assumption of Hemerik et al. (1990) that foraging activity is proportional to total activity within the nest. Secondly, in all cases we do not find correlations between all logical or structural subsections, suggesting that activity level is not linked directly to an environmental signal, global clock, or perfectly synchronized clocks within individuals. Thirdly, the occurrence of some correlations between subsections that do not share a common

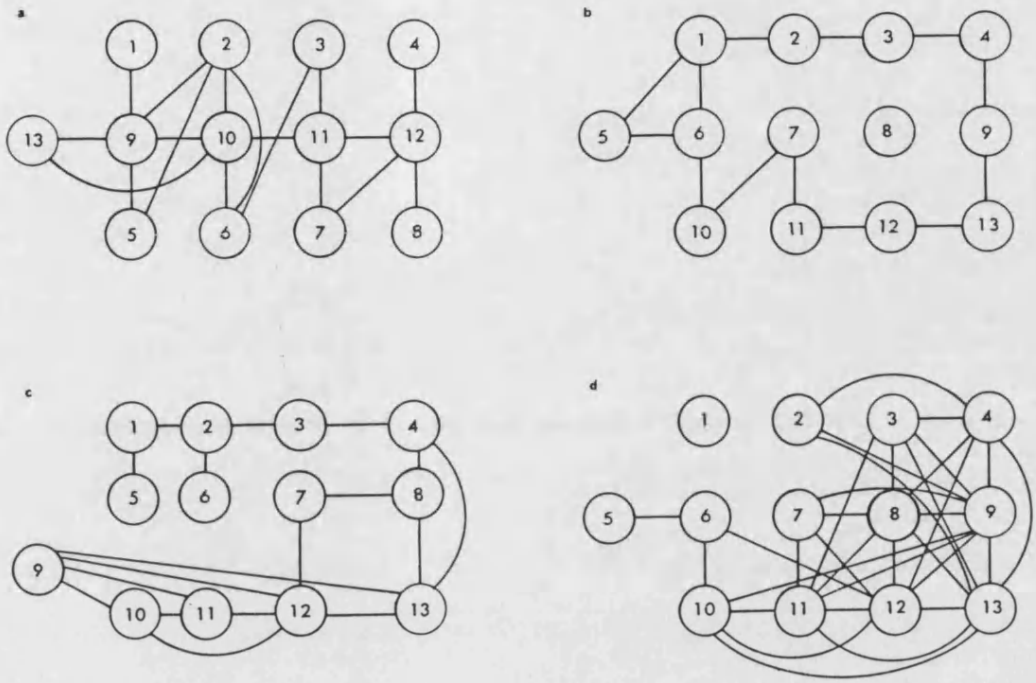

Figure 7.10: Maps of correlation between activity level in windows within nests. Windows are represented as numbered circles; the numbers refer to window identity as given in Figure 7.6. Lines that connect windows indicate positive correlation between activity levels in these windows. Positive correlation is judged to occur if the observed score of significant correlations summed over all days for each window pair exceeds the 95% upper confidence limit of the chance correlation expectation. a, 1POK; b, GRID; c, BOX; d, TUBRUN.

boundary suggests to some extent that not all the signal in subsection activity level is due to changes in position of individuals situated on the boundary between adjacent subsections.

The pattern of correlation appears to differ between nests. Correlations in the multichambered nest (Figure 7.10a) appear to be constrained by the physical barrier between chambers: activity within chambers appears to correlate with activity in the subsections of central passage to which the chambers open, and in general not to activity in other chambers. Where correlations do occur between chambers, they appear between chambers on opposite sides of the central passage. Also, activity in chambers below the passage tends to correlate with that in chambers to the right but not to the left above the passage. This reflects the precise physical structure of the nest (Figure 7.6 a): upper chambers open on the left whereas lower chambers open on the right. Hence lower chambers are closer (in terms of distance along connecting passage) to chambers above and to the right than those above and to the left.

Correlation patterns in the two single chambered *L. acervorum* nests (Figure 7.6 b, c) differ from the multichambered nest but are in general similar to each other. In both cases, a line of pairwise correlations connect the internal edge of the nest in a closed or open loop with the entrance. Interconnections between areas of the nest not directly abutting the nest wall appear to be rarer. This may reflect a tendency by individuals to move round the edge of the nest and periphery of the brood pile. The most strongly interconnected windows appear to be those that do not contain brood; the differences in correlation patterns within these two nests may reflect the different spatial distributions of the brood pile.

The pattern of correlations in the nest of *L. tubero-interruptus* (Figure 7.10 d)

appears to differ from that in structurally comparable nests of *L. acervorum*. This nest shows greater interconnectivity between subsections. Activity levels within windows representing the brood pile correlate to each other and to other areas of the nest. There is a suggestion that the activity of brood workers and those that reside at the entrance is more strongly coupled in *L. tubero-interruptus* than in *L. acervorum*. The greater correlation between activity levels in different parts of the nest might also explain why the time series of overall activity for *L. tubero-interruptus* (DIFRUN, TUBRUN) appear to contain less short term fluctuations (as indicated by fewer turning points in the data) than those of *L. acervorum*, resulting in a smoother appearance in the wave (Chapter 4; Appendix D.1).

7.2.4 Discussion

Multichambered nest: does activity level cycle?

Whilst the distribution of trough to trough intervals for overall activity within this structure does not appear to be a geometric decay after a fixed lag (WSCCS model), it does not seem reasonable at this stage to conclude that activity cycles do not occur. Visual interpretation of the whole colony activity timeseries or return maps do not suggest a qualitative difference to those for single chambered nests, although there may be some evidence that cycles are 'messier'. This experiment requires repetition with other colonies or other nest designs before we can place a higher degree of confidence in the conclusions drawn from visual interpretation or goodness of fit.

Similarly, ascertaining the existence of cycles within chambers requires further work, and it seems that manual measurements of individuals would be more

helpful here. There are a number of difficulties in interpretation of the digitized measures of activity, particularly in the case of small window size. Since the total level of activity within a single chamber is inevitably lower than that for the whole nest, the signal may be more prone to swamping by short term fluctuations. Hence from the digitized data alone, it would be imprudent to comment on the existence or otherwise of activity cycles at the 20 plus minute timescale.

The domination of high frequency cycles (of the order of 5-10 minutes) in the time series for individual chambers suggests frequent movement of individuals to and from chambers. Without measurements of individuals, we cannot deduce whether these individuals are brood workers active over several chambers, or individuals usually not involved in brood care that might reside in the central passageway. The occurrence of such interchange as evidenced also by correlations between chambers and passages may however suggest that even in physically constrained nests, a fair degree of interaction occurs between portions that could essentially function independently.

This may explain why overall cycles of activity could still be observed: cycles within chambers do not become decoupled as they are maintained in phase by interactions with a shared passageway. One might argue that overall cycles could be observed without any interaction; if the phases of individual chambers happen to coincide they may remain locked unless cycle length drifts considerably. However, we suspect that cycle length does indeed vary, as it appears to be distributed roughly as a geometric decay. Hence I would suggest that if overall cycles of activity do occur in a multichambered nest, they are maintained by mutual entrainment between loosely coupled oscillators (Winfree, 1980:112). That is, activity within an individual chamber may oscillate at some intrinsic frequency (dependent on number of ants therein), but is brought into phase with that in

other chambers through interchange of individuals along the passage.

Degrees of homogeneity

One feature that is marked in the analysis of spatial correlations of activity level is the relatively strong linkage within the *L. tubero-interruptus* nest. Portions of this nest appear to be more strongly coupled together than portions within the nests of *L. acervorum*. This result is in agreement with those presented in Chapter 5 in which *L. tubero-interruptus* 'task groups' were not found to differ with respect to individual cycle length, sleep or wake phase length, or frequency of contacts per sleep bout, in contrast to *L. acervorum*. Again, this experiment requires replication before much confidence can be placed in this conclusion. If we assume the conclusion to be an accurate reflection of the situation, we must consider why overall cycles of activity in *L. tubero-interruptus* do not seem to fit the WSCCS model more accurately than those of *L. acervorum*. We have strong reasons to suspect that the simple WSCCS model is inadequate in the case of *L. acervorum*, since activity parameters are not identical between individuals (Chapter 5). The same analysis of *L. tubero-interruptus* suggested that individuals differed less with respect to these parameters. A possible explanation lies in the lower speed of movement of these individuals (i.e., they differ in tempo: Oster and Wilson, 1978:281; pers. obs.; A.B. Sendova-Franks, T.R. Stickland, pers. comm.). Hence although activity might be locally strongly coupled (more pairwise correlations between neighbouring portions) the overall coupling may be lower, as signal propagation is slower across the whole nest. That is, although separate oscillators can mutually entrain, the speed at which entrainment occurs is not sufficient for overall cycles to fit the distribution expected from the WSCCS model. Hence in the case of *L. tubero-interruptus*, the assumption

that signal propagation is fast (less than 2% cycle duration) may be in error. It would be interesting to measure signal propagation rates in this species, using the technique described in Section 7.1, or by video analysis of individuals.

One overall feature of this analysis is the indication that the nests of both species are not homogeneous with respect to activity. Activity is not correlated just between nearest neighbours, suggesting that these correlations do not result from boundary interactions alone. The correlation maps may indicate the route by which interactions between individuals occur, and hence they may indicate the dynamics of information exchange within the nest. Hence for the multichambered nest, we may be witnessing information flow between subsections in accordance with the physical constraints of the nest design. In single chambered nests we may have some evidence for connections between the entrance and around the nest wall, or areas of the nest not occupied by brood, and eventually to the brood pile itself. This somewhat simplistic interpretation of the data is supported by observations of individuals: there is some tendency for ants at the entrance to circulate the nest wall, making contact with brood workers at the boundary of the brood pile (pers. obs.; A.B. Sendova-Franks, T.R. Stickland, pers. comm.) .

To summarize, this use of correlation to investigate activity in ant nests appears to yield some interesting results, although the strength with which we may wish to make interpretations is still open to question:

- there is a marked difference between the density and pattern of coupling in *L. acervorum* and *L. tubero-interruptus* single chambered nests, suggesting that *L. tubero-interruptus* workers tend to be more strongly interactive.
- The pattern of correlation in the multi chambered nest appears to reflect the physical structure closely, suggesting that activity is coupled through

physical mediation and that physical barriers constrain patterns of interaction.

- The pattern of correlation in single chambered *L. acervorum* nests appears to reflect the relative positions of the brood pile and nest entrance. Connections appear to 'run' from the entrance, along internal walls and then to the brood pile (or in the opposite direction). These connection might reflect patterns of movement and possibly also patterns of behavioural organization.

We might postulate that the activity correlation technique might be used as a limited automated assay for caste structure. Degree of activity coupling reflects strength of interaction between regions in space. This interaction results from the activity of workers, for which there is evidence of spatial fidelity; that is, individual workers tend to occupy particular zones within the nest, in *L. unifasciatus* (Sendova-Franks and Franks, 1992). Hence strength of coupling represents strength of interaction between groups of ants more or less restricted to spatial zones within the nest. Patterns of connectivity might map patterns of caste interaction, for instance if castes are restricted to particular locations (as they appear to be: Sendova-Franks and Franks, 1992), or if we can argue that connectivity within castes is stronger than that between castes (as considered by Wilson and Hölldobler, 1988). The repeatable automated nature of this technique would argue in favour of further work to investigate its use in answering questions concerning information exchange and behavioural organization in ant colonies.

Chapter 8

Discussion

8.1 Summary of Findings

In Chapter 2, I demonstrated the existence of a division of labour in colonies of *L. acervorum*, in that workers categorized on the basis of one behaviour performed other behavioural acts at different frequencies and possessed different maps of transition between acts. In particular, ants that did not leave the nest performed significantly more brood care. I developed an image analysis system (Chapter 3) capable of measuring positional change in ant colonies whilst maintaining a low level of noise, which could gather nearly seven hours of data on colony activity at a resolution of 1 minute. I utilized this system in Chapter 4 to obtain sufficient data to test models of activity cycle generation and allow accurate measurement of cycle length in nests of *L. acervorum* and *L. tubero-interruptus*. The results suggested that colony activity in both species tends to cycle, although cycles are not truly periodic (cycle length varies) and are not of regular shape or amplitude. Cycles in *L. acervorum* appear to be approximately 20 minutes in duration, those in *L. tubero-interruptus* roughly 35 minutes. Cycle length appears to be independent of colony size and brood to worker ratio.

In Chapter 5, I demonstrated that measurements of individual cycle duration largely conform to automated measures of colony cycles, but individuals of *L. acervorum* do not behave in an identical manner: the two task groups defined in Chapter 2 appear to differ in terms of cycle duration, those involved in brood care cycling at a slower rate. I also demonstrated that activity onset frequently follows physical contact from another ant, and that apparently spontaneous waking occurs rarely. In Chapter 6 I demonstrated that colony activity cycles are maintained in *L. acervorum* colonies deprived of food for a prolonged period, and that cycle length does not alter significantly under starvation. From measurements of individuals, there is evidence of a subtle colony response to starvation: brood workers decrease their activity through shorter active bouts and longer bouts of inactivity, whilst becoming more sensitive to potential foragers through an increased tendency to respond to physical contact. These results were supported by automated measures of activity correlation between different regions of the nest and foraging arena. In Chapter 7 I utilized the automated system to investigate patterns of activity coupling in nests of differing physical design and within regions of the nest. I demonstrated that activity is more strongly coupled within nests of *L. tubero-interruptus* than those of *L. acervorum*, and that the pattern of coupling may reflect features of nest structure and organization.

Individuals appear to synchronize their activity as a result of positive feedback in the activation phase, whereby active ants stimulate inactive ants by physical contact (Chapter 5). This process leads to cycles in activity at the colony level; however these cycles are not truly periodic (Chapter 4). The lack of periodicity and variation in amplitude appear to result from several factors:

- the probabilistic nature of individual activity onset leads to variation in colony cycle length (Chapter 4, Tofts, 1990a).

- differences between individuals result in a complex activity wave at the colony level: in *L.acervorum* two task groups cycle at different frequencies (Chapter 5) but are coupled together to some extent (Chapters 6 and 7).
- Individuals are not accurate time keepers; they may become active 'early', or fail to respond to others and become active 'late' (Chapter 5).

The activity cycles are however quite robust to a variety of manipulations: cycle length is independent of colony size, brood to worker ratio (Chapter 4), food availability (Chapter 6), and to some extent the physical design of the nest (Chapter 7). These results point to the rejection of two models (Goss and Deneubourg, 1988; Hemerik et al., 1990) concerning cycle mechanism. The results generally support the model of Tofts (1990a) although differences in behaviour of individuals suggest that the first version of the model is too simplistic. Robustness of cyclicity appears to result from aspects of the synchronization mechanism:

- signal propogation is relatively fast: a single individual may wake all others in a time scale that is short compared to cycle duration (Chapter 7).
- although individuals 'wake spontaneously' with low probability (Chapter 5), the probability of colony activation is high as only one individual is required to wake for activity to propagate throughout the nest (Tofts, 1990a).

I have demonstrated that an automated system of data collection may be used to investigate the simple behavioural parameter of activity timing in colonies of ants. However, it is also apparent that such a system might be used to investigate broader aspects of behaviour, such as some aspects of organization in social insect colonies. For instance, I have exploited image analysis to investigate patterns of activity coupling between regions of the nest, demonstrating that colony activity

is not proportional to activity within the nest (Chapter 4) but becomes more closely linked under food deprivation (Chapter 6), and that patterns of activity coupling differ between species and within species in relation to nest physical design and spatial arrangements within nests (Chapter 7).

Clearly, biological interpretation of these results also requires measurement of the actions of individuals, and will entail manual observation. However, the two methodologies may complement each other to forward our understanding of individual and social behaviour.

8.2 Other Approaches to Cyclicity

8.2.1 Biological Rhythms

Rhythms, that is, the recurrence of events at more or less regular intervals (Aschoff, 1981) occur extensively throughout biological systems. Although many of these rhythms are self sustaining without environmental input (i.e., they are endogenous), in most cases there is also a clear exogenous component in the systems behaviour, and hence clear avenues for functional explanation of those rhythms.

Circadian rhythms in physiology and behaviour are widespread in both marine and terrestrial organisms from unicells to mammals. The mechanism underlying maintenance of these rhythms varies, from one endogenous pacemaker entrained to physical stimuli of light or temperature, to two or more endogenous pacemakers entrained to physical rhythms that may be coupled together to a lesser or greater extent (dinoflagellate: Hastings, 1960; invertebrates: Truman, 1972; Page, 1978;

Hudson and Lickey, 1977; Koehler and Fleissner, 1978; birds: Zimmerman and Menaker, 1979; mammals: Hoffmann, 1971). In the absence of light or temperature rhythms, these systems maintain rhythmical behaviour at a slightly higher or lower frequency than the 24 hour cycle (Aschoff, 1979; Pittendigh and Daan, 1976). The precise mechanism of endogenous clocking components is unclear; in some cases individual cells may be responsible, whereas in other interactions between cells or groups of cells may lead to cyclicity (Fentress, 1976; Moore-Ede and Sulzman, 1981).

Circadian rhythms have obvious adaptive advantages, for instance in control of activity to favourable periods of the day or night (Daan, 1981). Further their initial evolution is not difficult to imagine: the existence of regular cycles in light levels resulting from the motions of the planets and stars has enabled the development of systems capable of responding to these predictable events, and hence adjusting their behaviour to suit environmental cycles (Pittendrigh, 1981a). Similarly, seasonal fluctuations necessitate annual cycles in behaviour for many organisms, and timing strategies have evolved that utilize predictable cycles in physical factors such as day length. Indeed the mechanisms of circadian control are frequently utilized for annual control (for example, insect and vertebrate photoperiodism: Saunders, 1981; Hoffmann, 1981) and enable preemptive response, such as in the timing of reproduction, hibernation (for example ground squirrels: Pengelley and Kelly, 1966) and migration (Gwinner, 1971).

Circatidal rhythms present another case were clear adaptive advantages can be cited to account for their occurrence; organisms in the intertidal zone must restrict their activity to times of high or low tide. However, the precise timing and duration of tides is less predictable than daily or annual light cycles owing to the complexity of factors involved. In this respect, it is interesting that tidal organ-

isms appear to possess a variety of rhythm maintaining mechanisms, and that the occurrence of strong endogenous components seems to be less common. Although clear endogenous components exist in some species (for example Fiddler crabs: *Uca pugnax*; Enright, 1965), activity in others appears to be regulated largely by exogenous stimulus (for example *Uca urvillei*; Lehmann et al., 1974). For *Uca urvillei* it appears that tidal factors control the duration of resting periods, but under constant conditions the duration of activity and rest bouts is determined by a fixed probability of transition (Lehmann et al., 1974; Kaiser and Lehmann, 1975). Such a timing mechanism may be advantageous in that individuals can react quickly to unexpected situations (tidal surges or storms).

A final class of behavioural rhythms known as 'short term' (Daan and Aschoff, 1981) concern bouts of activity and inactivity that alternate with higher frequency than once per day. Among cycles cited in this group are the roughly two hour cycles of activity in rodents (*Microtus agrestis*; Lehmann, 1976; *Sorex araneus*; Crowcroft, 1954), and the four hour grazing cycles in ruminants (Hughes and Reid, 1951). These rhythms possess characteristic variable period length, and do not correspond to any known environmental periodicity. Daan and Aschoff (1981) consider possible causal mechanisms and functions of short term rhythms, and conclude that neither are well researched. Although cycle length appears to be related to metabolic rate in some instances, there is little evidence of a causal link. Davis (1933) suggested that the process of stomach filling alternating with digestive pauses might be responsible for rhythms in voles, but continued periodicity under food deprivation suggests otherwise (Daan and Slopsema, 1978). Functional explanations for short term rhythms generally involve that such cycles form "stable elements in the temporal organization of behaviour" (Daan and Aschoff, 1981), which may be adapted with respect to the external world (for example, predator avoidance) or the internal milieu (for example, appropriate

timing of physiological events), such that the pattern observed reflects the optimal pattern of feeding and rest for that species.

The pattern of activity in ant colonies may be considered as an example of short term rhythms. Cycles in activity are of variable length, are not related to any known environmental rhythm and occur at frequencies much greater than once per day. The underlying mechanism might be similar to that in *Uca urvillei* tidal rhythms: onset of activity or inactivity is probabilistic, and rhythms result from a preferred duration to one of these phases (inactivity). Unlike *Uca urvillei*, where the rest period is determined by an exogenous factor (tide), the rest period of individual ants appears to result from an endogenous, albeit inaccurate clock mechanism. Cycles within the colony are more accurate as a result of interactions leading to synchrony among clocks. The question of cycle function has yet to be answered as in the case of other short term rhythms. It is of course possible that colony activity cycles afford no function (see Section 8.3.3). In Section 8.3 I discuss a number of possibilities that address aspects of the temporal organization of behaviour in colonies.

One of the problems in relating colony activity cycles to other biological rhythms concerns the nature of endogenous and exogenous stimuli. For circadian rhythms, there is a clear exogenous component, namely, environmental features such as light intensity. If the rhythm is not purely the result of response to the stimulus (it persists in the abs ence of environmental rhythm) an endogenous component can be suspected. Considering activity in the colony as a whole, there appears to be no exogenous stimuli. However, from the perspective of an individual ant, contact from other ants may be considered as a potent exogenous force. Nevertheless, some form of endogenous mechanism (within individuals) also exists since the response of individuals to contact is related to their own phase (the time during

rest at which contact occurs). Hence it might be more appropriate to consider the colony clock as analagous to a pacemaker organ within organisms, behaviour of which results from interactions between cells with certain response thresholds and relaxation periods of their own.

8.2.2 Dynamics of individual activity

The activity profile of individual, isolated ants of *Leptothorax allardycei* has been studied by Cole (1991a-c, 1992) with respect to activity cycles. Cole (1991a) reported that individual ants did not exhibit cyclical activity patterns, becoming "active spontaneously, but at no particular interval". However, Cole (1991b) concludes that the activity of individual ants is not unpredictable, since virtually all the bouts ranged between 0.5 and 1.5 times the mean bout length. In Cole (1991c) the activity of single ants is described as chaotic, since the activity timeseries possess an attractor of (small) noninteger dimension. These differing conclusions derive from three different analysis techniques. The first involves spectral analysis of timeseries from digitized measures (1991a), the second involving goodness of fit to frequency distributions from observations of video recordings of ants (1991b), and the third employs methods of chaotic attractor construction and dimension measuring from digitized data (1991c). In the 1991a, b studies, individual ants were removed from the nest and isolated in a separate chamber 30 minutes before filming commenced. The experimental conditions are not reported in the 1991c study, but I assume that they are identical to those of the 1991a study since in at least one case the same timeseries is analysed in both papers. In the case of the video analysis (1991b), ants were isolated into chambers 15 $\times$ 15 mm in size.

Although I too attempted studies of isolated ant activity, I have not presented the results in this thesis owing to difficulties in their interpretation. A question which I suggest is of fundamental importance concerns what such isolated ants can be regarded as representing.

Isolated ants are not identical to individual ants within a colony, since they are deprived of context: they are unable to interact, receive (or provide) a stimulus from (or to) other individuals. Thus, we might expect isolated ants to exhibit behaviours or patterns of behaviour that are uncharacteristic of individuals within a colony, since removal from familiar colony interactions and odour into novel surroundings might induce stress responses.

For this reason, even the most simplistic interpretation we would wish to make may be open to doubt. Cole (1991a-c) shows that the activity patterns of isolated ants differ from those of colonies: they are less predictably periodic, and activity bouts tend to occur less frequently than in intact colonies. These results are consistent with my own observations. It is tempting to conclude from this that individual ants do not possess an intrinsic, accurate activity clock. On the basis of these experiments alone, where individuals were recorded 30 minutes after being isolated, this conclusion should be viewed with caution since the experimental manipulation may have upset any intrinsic timing mechanisms. Similarly, measurments of the activity of two or so individuals removed from the colony and confined to a novel environment (1991a, b) may be difficult to interpret.

I attempted to measure the activity of individuals and small groups that had been isolated but allowed to acclimatize for several days. On the basis of preliminary experiments with intact colonies, even this period of acclimatization may not have been sufficient. In any case, the individuals frequently died within a week

of isolation, again suggesting that colony context is an important aspect of ant identity.

Whether natural selection is regarded as acting on individuals (Hamilton, 1964; Maynard Smith 1964), or at the colony level (Wilson, 1975; Colwell, 1981; Wilson and Sober, 1989), it cannot act on isolated worker ants. Fossil evidence suggests that ant species with distinctly modern characteristics that are likely to have included eusociality had undergone adaptive radiation no later than the early Tertiary (Wilson, 1987). It is thus not surprising that isolated worker ants exhibit aberrant, "mal-adaptive", or "chaotic" phenotypes in species in which such phenotypes (behaviour under isolation) have not been expressed for at least 65 million years.

8.2.3 Chaos in behavioural dynamics

Cole (1991c) concludes that individual ant activity exhibits low dimensional chaos, in contrast to the rhythmic activity of whole colonies. He suggests that colony context allows order to be imposed on otherwise chaotic processes. Individuals behave in a chaotic manner as a result of complex interactions between factors that determine activity, such as genes, physiology and environment. Hence individual behaviour is deterministic rather than random or the result of chance occurrences (Cole, 1991c). For reasons stated in the previous section, we might question whether demonstration of chaos in experimentally isolated worker ants can be cited as evidence for chaos in the behavioural dynamics of other organisms. This apparent chaos may represent a stress response to novel environments when an individual is removed from the colony context in which its behaviour is organized. As yet, it has not been neccessary to conclude that the behaviour of solitary organisms is chaotic. Simpler approaches to the study of temporal patterns of ac-

tivity seem to have sufficed (for example Reynolds et al., 1986; methods reviewed in Fagen and Young 1978), with the advantage that the effects on a behaviour of physiology, environment and genes can be investigated mathematically and experimentally. Indeed, there is good reason to suspect that interactions leading to chaotic outcomes will be selectively avoided, as chaotic systems are prone to sensitive dependence on initial conditions. Hence slight differences in physiological or environmental conditions could result in major differences in patterns of behaviour, in situations where stable or slightly altered patterns would be more appropriate. It seems more plausible at present to conclude that chaos in isolated ant activity is the result of removal of the organism from the context under which selection on behaviour occurs (the colony) than that it occurs commonly in the behaviour of solitary animals and has been overcome by the constraints of social interaction.

8.2.4 Rhythmicity and Phase Response

Cole (1991b) utilizes the phase response methods of circadian rhythm research (Winfree, 1980:82; Pittendrigh, 1981) to investigate the mechanism of interactions between individuals and its relationship to colony level cycles of activity. A phase response curve is generated by plotting the phase shift effects of a stimulus given to a subject at various points in the phase. Cole investigates the phase shift resulting from stimulus (physical contact by another ant) given at various times to a focal ant. His experiments involve isolating two ants from a colony, and measuring the intervals between onset of inactivity and receipt of stimulus, and between stimulus and onset of activity. The arguments presented above, concerning whether isolated individuals can be dissected from the colony without altering their basic patterns of behaviour also apply here. Further, in order to

generate a meaningful phase response analysis, Cole must assume that individual isolated ants do possess an underlying rhythm of activity. This conclusion seems to be somewhat at odds with his other conclusions drawn in the 1991a, c papers.

Cole demonstrates that the effect of stimulation is always to advance the phase of the recipient, as opposed to delaying it. Hence stimulated individuals reach the active phase of their cycle faster than when not stimulated. He demonstrates that stimulation does not cause transient activity that leaves the oscillation unchanged, nor does it change the period of the oscillation in successive cycles. He also shows that the amount of phase shift caused is dependent upon the time at which stimulation occurs; stimuli early in the phase of inactivity have relatively low phase shift effect compared to stimuli received later in the inactive phase. His data are thus consistent with my own (Chapter 4), showing that inactive ants can become active after receipt of physical contact, but do not always do so.

Coles's use of phase response analysis for this system is limited, since he ignores the problem of multiple stimuli. An individual ant within a colony frequently receives more than one physical contact during any inactive phase (in some instances, 30 or more: see Figure 5.7). Cole measures the time intervals relative to the first stimulation only, and does not report whether further stimulation occurs in any given cycle. The apparent phase shift caused by stimuli early in the inactive phase might also be explained as an immediate response to a later stimulus, the early stimulus being ignored.

Hence, Cole's findings may also be consistent with the model of Tofts (1990a; see also Tofts et al., 1992), in which stimulation before a certain point in the cycle is ignored, but leads to activity onset if it occurs after that point (after the sleep phase in model 1 or the deep sleep phase in model 2; Figure 5.8).

8.2.5 Multiple oscillator theory

As Cole (1991a, b) points out, ant colonies can be considered as a population of excitable subunits, as analysed by multiple oscillator theory (Winfree, 1967; Pavlidis, 1969). Each ant can be considered as an oscillator that is to some extent coupled to other oscillators (ants) via interactions between these units. A variety of colony level dynamics may be observed, depending on the strength of coupling between units, and the variation in frequency of oscillation between independent oscillators (Cole, 1991b).

If ants are considered to be perfectly accurate clocks (that is, waking with probability 1 at time t), a pair of ants can be written:

$$x = 1 + f(x, y) \qquad \text{(i)}$$
$$y = 1 + f(y, x)$$

That is, the change in phase x of ant 1 is dependent upon its own phase plus some interaction $f(x, y)$ from its partner.

Winfree (1967, 1980:112) discusses whether populations of oscillators with the same fundamental frequency, or slightly different frequencies, may mutually entrain or even synchronize as a result of interactions. For pairs of oscillators (clocks) which have some effect on each other's phase (interact and cause phase resetting), he shows that mutual entrainment (oscillating at a common frequency) and synchrony (oscillating in step; phase locked) can occur, but are not the only possible outcomes. By a procedure of iteration, he argues that populations of more than two clocks can behave similarly (Winfree, 1980:117). His argument

proceeds by considering a population in which two clocks entrain and synchronize, and thereby can be represented as a single clock. Repeated individual clocks may then be entrained to the amalgamated clock by repetition. The behaviour of multiple clocks is considered by verbal argument owing to the difficulties in mathematical analysis of more than a single couplet of equations (i) above. It is not clear that the argument holds if entrainment and synchrony cannot be considered to occur serially. If the starting population consists of many unsynchronized clocks, it may not be possible to ignore the interaction terms of other units in the population that are not part of the clock plus amalgamated clock current focus.

In any case, Winfree (1980:117) concludes that such a population may mutually synchronize or it may split into separate subpopulations of entrained clocks, or even actively avoid synchronization by mutual repulsion. The population, if in sychrony, may run at any period, even faster than the fastest element, or slower than the slowest. Pavlidis (1969) uses this phenomenon to show how rhythms on a circadian time scale may be generated by biochemical oscillators, if the oscillators are coupled under some negative feedback conditions.

The use of multiple oscillator theory to analyse the dynamics of ant activity is further complicated by our suspicion that individuals are not perfect timekeepers, but are likely to become active (terminate a cycle) probabilistically after some lag time. Hence a pair of such ants can be written:

$$x = D(t_1) + f(x, y)$$
$$y = D(t_2) + f(y, x)$$

Hence the change of phase x is dependent upon the interaction $f(x, y)$ and the

time of activity onset t_1, where t_1 is an element of the stochastic distribution $D(t_1)$. These equations are the continuous representation of the discrete WSCCS model (Tofts, 1990a). They consist of a fixed element $f(x, y)$ and a stochastic element $D(t_1)$, and are extremely difficult to analyse (Arnold, 1974; Doering, 1991). Hence it is unlikely that models based on these principles will yield clear predictions, given the difficulty in predicting precise outcomes for multiple oscillators without stochastic elements (Winfree, 1980:112).

8.3 Adaptive Significance of Cycles

8.3.1 Foraging control

Hemerik et al. (1990) posit that cyclical colony activity may be linked to colony energy levels, and thereby might be involved in guaging energy levels and facilitating appropriate foraging responses. As previously discussed (Chapters 4 and 6) e mpirical evidence in support of their model is lacking, indeed most data provided in this thesis tends to contradict the predictions of Hemerik et al. Also, I have argued (Chapter 4) that the mechanism proposed by Hemerik et al. to account for foraging regulation may not be advantageous, since it may not allow the colony to respond to diminishing energy levels at suitable speed to alleviate the risk of starvation. This argument is essentially similar to that of Krebs and McCleery (1984) concerning the problems of satisfaction strategies in unpredictable environments.

Herbers (1981a) analyses state based models of feeding based on satisficing asumptions for a variety of theoretical organisms. She concludes that such thermostat models of feeding may lead to a high proportion of inactivity in organisms, as

witnessed (among others) in social insects. However, she points out that the models analysed are not good approximations of social organisms, in which food may be considered to be stored (in the form of brood).

At present, it seems difficult to link activity cycles to foraging strategies, especially since the mechanism proposed by Hemerik et al. (1990) requires that individuals have knowledge of global phenomena, and that the time scale for variation in colony energy level may be much greater than that over which cycles operate.

In Chapter 6 I note some possible responses (on the basis of observations of individuals, and digitized data from regions of the nest) that may lead to increased allocation to foraging whilst allowing net energy conservation under starvation stress. These mechanisms, if they exist, do not appear to be related to activity cycles *per se*. They may however bear some similarity to Seeley's (1989a) findings for bees, in which waiting time for returning foragers offloading food at the nest appears to determine future foraging rate.

If parallels exist between honeybee and leptothoracine foraging rules, then colony level patterns may change in the way witnessed under starvation. As the proportion of demand (from broodworkers) to supply (from foragers) increases, broodworkers may queue away from the brood pile to receive food, in contrast to a satiated nest in which foragers approach the brood pile to find recipients for the supply. Such a system would involve individuals operating simple rules based on negative feedback between supply and demand, as a result of their own direct sampling experience. Although we may observe colony level patterns, for instance in space utilization or the position of zones of interaction within the nest, these patterns are the result of individual sampling and interactions, and would not

appear to require particular temporal patterns of activity for their operation.

8.3.2 Sampling rate and information exchange

Franks and Bryant (1987) and Franks et al. (1990a) suggest that synchronization of activity may allow more efficient information exchange within ant colonies by improving sampling rates of individuals. Considerations from computer science (e.g., Mead and Conway, 1980: 221,238) would tend to support this suggestion. It is often essential to achieve synchrony of action of subcomponents by implementation of a clocking mechanism to allow processing to occur correctly; asynchronous action may result in operations being performed on the wrong (out of date) information.

In ant colonies, we may suppose that information concerning a variety of issues must be sampled by individuals, in order to guage some colony parameters. For instance, individual foragers may sample the nutritional state of other individuals in order to decide whether they should forage. If food levels are high, foraging may not be appropriate since it entails use of energy resources to maintain forager activity, and possible risk of death of the forager by predation (Herbers, 1981a). Hence accurate information on food levels within the colony would appear to be advantageous.

Assuming that information cannot be sampled from inactive ants, synchronized activity can lead to increased accuracy of sample based information. Improved accuracy may be considered in two ways. Firstly, if a threshold sample size is required in order to make a decision, this can be obtained more rapidly during synchronized activity. This will occur as the average search time to locate an

active ant to sample decreases, since less time is lost approaching inactive ants that cannot be sampled. By reducing the time required to achieve the sample threshold, the summed information is less likely to be out of date (Franks and Bryant, 1987). Also, since search time is reduced, the energetic costs of searching are reduced. Secondly, we may postulate that there is a threshold cost to sampling, beyond which it is advantageous to make a decision rather than sample again (due, for instance, to diminishing returns of information reliability and increasing energetic cost). In this case, if the cost is considered to be proportional to the total time spent sampling, then for a given sample bout duration, the sample size can be increased under synchrony as a result of reduced search time between samples.

Hence synchrony may improve the reliability of information obtained in a given sample bout. However, it can be argued that the benefits of synchrony do not outweigh the costs of reduced periods during which information can be exhanged; sampling cannot occur during the inactive phase. For foraging, and other problems concerned with daily maintenance of the colony, it is likely that major changes in colony state do not occur at a rate higher than the frequency of sampling bouts (activity approximately every 20 minutes). It may be possible to develop models to investigate suitable time intervals for information exchange, for example with respect to foraging success rates, based on the marginal value theorem (Charnov, 1976).

Certainly some aspects of information exchange are not plausibly enhanced by synchrony. For instance, potentially catastrophic threats which may occur suddenly such as nest invasion by predators would not elicit a quick response if workers were simultaneously inactive when they occured. However, leptothoracines do not appear to be perfectly synchronized; in particular the activity of individuals

outside the nest does not correlate strongly with activity levels inside the nest. On the basis of digitized measures of activity, complete synchronization of inactivity within the nest is also seldom achieved. It is likely that colonies possess signal mechanisms that override the normal system, such as alarm pheromones (Hölldobler and Wilson, 1990:260). Hence provided that some individuals are responsive to immediate threats, the colony will be able to respond from the inactive state.

8.3.3 Synchrony and cyclicity as an epiphenomenon

The arguments given concerning sampling efficiency under synchronization (see below also) suggest adaptive advantages for synchrony, but do not concern periodicity. Further, it is not clear that these arguments can be used to explain the evolution of synchrony from an asynchronous state. For instance, if activity is partially synchronized, such that some ants are in synchrony but others are active randomly, then the average sampling search time may be no lower than when all individuals become active at random. This might occur since individuals that are not in synchrony will take longer than average to find active individuals to sample. In parallel with arguments concerning the evolution of sex, features that may allow for its maintenance (and in this case, improvement), may not explain its initial evolution (Maynard Smith, 1978).

Cole (1991a, 1992) argues that cyclical activity patterns may have evolved as an epiphenomenon, resulting from selective pressures acting on basic behavioural responses of individuals. Cole suggests that selection may act on the level of spontanaeity of waking of individuals and on the degree of coupling between individuals, that is, their propensity to respond to stimulation. Synchronized

activity, and possibly periodicity, might result for certain levels of these characteristics. He suggests that colony level selection may have selected values for these parameters that yield favourable levels of activity. Clearly, if colony activity level is the sole trait under selection, periodicity is not the only outcome; activity levels could also be adjusted by selection on individual activity levels without coupling. Hence individuals could become active spontaneously but the frequency of waking or the length of active bout may be under selection.

However, Cole's view of activity cycles as an epiphenomenon cannot be discounted. It is possible that high propensity to react to stimuli has been selected for reasons unrelated to periodicity or synchrony in activity. For instance, strong coupling may allow fast resonse to threats to the colony, such as flooding and invasion of predators, whilst allowing conservation of energy through periods of inactivity (Hatcher et al., 1992b). A tendancy of individuals to become active when physically contacted by others will result in some degree of synchrony, and hence colony activity bouts. Again, periodicity will not neccessarily result, although colony activity levels may be interpreted as cyclical as a result of synchronization of individuals.

8.3.4 Synchrony and mutual exclusion

In the preceeding section, I discuss the possibility that synchrony arises as a byproduct of selection for responsiveness of individuals to stimulation by others. Given that synchrony evolves under these conditions, it may be advantageous to the colony to maintain and improve synchrony. In Section 8.3.2, I discussed one possible advantage, namely that synchrony may enable improved information exchange as a result of increased access of individuals to others from whom infor-

mation can be actively sampled. Synchrony may also allow improved information exhange through another route, involving the passive exchange of information that may occur as a result of spatial arrangments of individuals within the nest. This possibility, discussed below, is also presented in Hatcher et al. (1992a,b).

One situation in which positional cues may be utilized as information is the problem of allocating resources to brood. The brood to worker ratio in leptothoracines frequently lies above unity (Han, 1989; Headley, 1943), so some brood items are likely to remain untended for a considerable time if items are chosen at random. Successful growth and development of brood depends upon adequate feeding and regular cleaning to prevent the growth of fungi (le Masne, 1953; Maschwitz et al., 1970). Brood items that remain untended for some time may die, or suffer a diminished growth rate and prolonged time to pupation. We would expect nurse workers to distribute care frequently and evenly among brood items in order to maximize brood survival. If food availability is low, this argument may not apply, since it may be more advantageous to care for some items and ignore others, so that at least some are fed sufficiently. When survival is not limited by the availability of forage, an even distribution of care may maximize brood survival.

A more uniform distribution of care could be achieved if nurse workers were active simultaneously, and individual brood items are not tended by more than one worker at the same time. Leptothoracine brood is gathered into clusters; this spatial arrangement appears to limit the number of workers that can access a given brood item simultaneously (le Masne, 1953; Franks and Sendova-Franks, 1992).

Hence the spatial arrangement of brood combined with the temporal arrangement of worker activity will lead to mutual exclusion between nurse workers; the

presence of one nurse worker on an item excluding the presence of others.

To compare brood tending in the case of synchronized activity versus that of asynchronous activity, I take an accounting period, during which each ant is active once; in the case of *L. acervorum* I would take this period to be 20 minutes. For simplicity, I assume that an ant can tend only one brood item per active phase.

When worker activity is synchronized, and mutual exclusion occurs between nurse workers, the proportion $P_{exclusion}$ of brood tended in any accounting period becomes:

$$\text{for } A < B\text{: } P_{exclusion} = \frac{A}{B} \qquad \text{(i)}$$
$$\text{for } A >= B\text{: } P_{exclusion} = 1$$

(A is the number of nurse workers; B is the number of brood items).

Thus $P_{exclusion}$ increases linearly as the worker to brood ratio rises (Figure 8.1); clearly when A exceeds B all brood items will be tended in the accounting period.

If workers are active asynchronously, mutual exclusion will not occur even if the spatial distribution prevents simultaneous access. In this case, items are chosen at random during any accounting period; the proportion P_{random} tended is as follows:

$$P_{random} = 1 - [\frac{(B-1)}{B}]^A \qquad \text{(ii)}$$

(A and B as above).

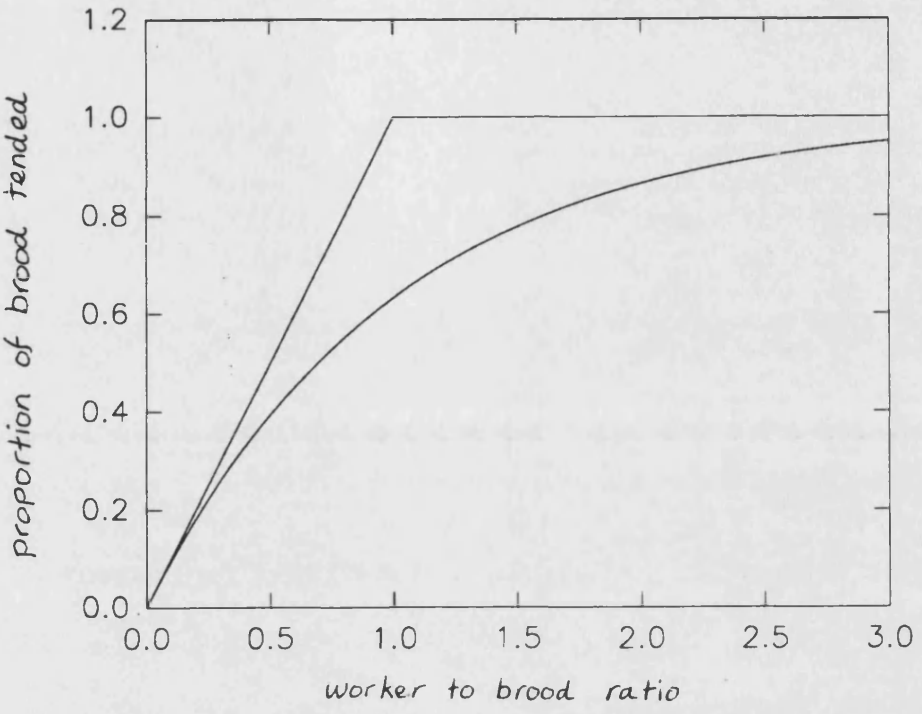

Figure 8.1: Relationship between the proportion of brood tended in an acounting period and the worker to brood ratio. The line $P_{exclusion}$ represents the relationship assuming that mutual exclusion operates as in Model (i). P_{random} represents the relationship when brood items are chosen at random (Model (ii)).

Hence P_{random} increases exponentially as the worker to brood ratio increases, but remains below $P_{exclusion}$ for all worker to brood ratios (Figure 8.1). The derivation and behaviour of (ii) follows Nicholson's "competition" curve (Nicholson, 1933) for the random encounter of hosts by parasitoids. Similarly, (i) is equivalent to the model presented by Hassell (1978:12) for host encounters by parasitoids that cooperate to avoid revisiting previously searched areas. In effect, mutual exclusion between ants (or parasitoids) represents an automatic and effective exchange of information on task allocation (or oviposition).

In order to test this model directly, it will be neccessary to examine a large sample of brood items simultaneously over a number of accounting periods (i.e., activity cycles) to test the fit of the random and exclusion models to the observed proportion of brood tended. Since I have yet to find an experimental manipulation that leads to disruption of cycles, it is not possible to compare brood tending between a synchronized nest and an asynchronous one. To confirm that regular brood tending confers adaptive advantage, it would also be neccessary to follow the survival of brood items tended at different rates through to eclosion. A number of factors are likely to confound the precise fit of the data to the optimal exclusion model presented; for instance, nurse workers may tend more than one brood item per activity cycle; individuals may have a preference for particular locations within the nest (Sendova-Franks and Franks, 1992); and brood items of different age are distributed unevenly with respect to location and nearest neighbour (Franks and Sendova-Franks, 1992). Also, dominance interactions between workers within nests of leptothoracines (Franks and Scovell, 1983; Cole, 1981) may further structure brood care activities in time and space. Nevertheless, some degree of synchronized activity amongst nurse workers will automatically result in some level of mutual exclusion on brood items, i.e., choice of brood items for tending will not be entirely random, consequently the proportion tended per

activity period will be greater than that predicted by the random model.

Hence, for some problems of colony maintenance, synchrony may allow efficiency in resource allocation by reduction of task repetition.

Alternatively, synchrony may be seen as reducing the variability of outcome of performing given tasks. In the example above, variation in resource allocation to brood is reduced as a consequence of mutual exclusion. Rubenstein (1982) in a theoretical analysis of foraging strategies, has shown that it may be advantageous for individuals to forage in a habitat with lower variation in reward rate, even if the mean reward rate is lower than that in a variable habitat. This appears to be the case when increased allocation is linked to diminishing returns, and so might apply to the case of brood care, where increased attention to brood items is unlikely to pay back linear dividends in colony growth rate. In some senses, synchrony might be viewed as a route to increasing effective colony size, and thereby reducing variability in success of colony level task performance.

This argument has been couched with reference to brood care, but synchrony may reduce redundancy in other tasks, possibly by improving efficiency of information exhange (passive or active), the outcome of which determines allocation of workers within and between tasks (see section 8.3.6). Mutual exclusion may also allow efficient transfer of metabolites from larvae to queens and eggs. In *Solenopsis invicta* large larvae appear to act as metabolic reserves (Tschinkel, 1988); uniform attendance of brood items might result in higher yields of material from larvae.

8.3.5 Levels of selection

In the preceeding discussion, I have left the precise mechanism of evolution (i.e., level at which selection is viewed as acting) vague. This is because there is no conflict between individual level and group level arguments for the various adaptive advantages suggested. Some authors (e.g. Wilson ,1992; Cole, 1991a) prefer to discuss cases from the perspective of the group (in this case, colony), whereas others (Maynard Smith 1964; West Eberhard 1975, 1981) utilize arguments based on advantages to the individual. I would agree with Maynard Smith (1982a) and Grafen (1984) that forms of selection refered to as "kin group" (Wilson and Colwell, 1981) or "group" (Wade, 1985) are in essence kin selection. From the focus of an individual ant, kin (individuals more closely related than the population average) happen to be those individuals that inhabit the same colony. In the previous discussion, it is unneccessary to invoke group selection (sensu Wynne Edwards, 1962), since the models suggested do not indicate a conflict of interest between individuals and the colony as a whole. That is, individuals with behavioural patterns that improve the efficiency of foraging, brood care, or information exchange will exhibit higher inclusive fitness (raise more kin in the case of sterile workers) than those that do not possess such attributes.

For instance, let us consider the case of selection for response to physical contact (Section 8.3.4), as a result of improved colony response to threat. If responsive individuals are more able to avoid death, and can thereby contribute further to investment in sexuals, their inclusive fitness will be higher than that of unresponsive individuals. To the extent that they are related to these sexuals, the genes ultimately governing responsiveness will spread in the population, all other things being equal. The argument is similar to that concerning whether selection acts on individuals or genes (Williams, 1966; Dawkins, 1976; Sober, 1984). Al-

though selection may appear to act on colonies (colony response to disaster), it is the behaviour of individuals that determines colony level outcomes. Colonies may be considered as vehicles for the transmission of genes possessed by individuals (Dawkins, 1982). Generally, genes increase in the population as a result of increased inclusive fitness of their vehicles. However, the selective interests of genes and their vehicles are not always parallel (for example, junk DNA; Dawkins, 1982).

If worker ants are able to overcome sterility, which in some cases appears to occur, conflict may arise between queens and workers over the production of males. Individuals that are able to reproduce may conflict with each other, resulting in dominance heirarchies (Franks and Scovell, 1983; Cole, 1981). Reproductive individuals may improve their inclusive fitness by avoiding risky tasks such as foraging, remaining in the nest where they can lay eggs. This situation may result in apparent temporal polyethism (West-Eberhard, 1979), if ovaries atrophy with age. Hence younger potentially reproductive individuals may remain in the nest and carry out brood care, whereas older workers that cannot reproduce contribute to the fitness of kin by undertaking foraging (West-Eberhard, 1979, 1981; Sudd and Franks, 1987:76). It is also possible to account for the direction of polyethism without reference to the direct reproductive interests of individuals. Older workers represent a "disposable caste" (Porter and Jorgensen, 1981). Other things being equal, they will expire sooner than young workers, so it is advantageous to the colony (and to individuals via kin selection), that those most likely to die are employed in the riskier tasks.

In the next section, a general model for the evolution of synchrony is introduced, which involves features discussed by West-Eberhard (1979) and Porter and Jorgensen (1981). I attempt to indicate how choices made by individuals to maximize

their own fitness (direct or inclusive) may enable efficient colony function.

8.3.6 Synchrony and worker allocation

Considerable evidence supports the notion that workers of some ant species, including leptothoracines, can lay unfertilized eggs that develop into males (Bourke, 1988a,b; Cole, 1981, 1986; Choe, 1988).

The scale of this phenomenon is not clear, since eggs may be layed for trophic purposes (Passera et al., 1968; Voss, 1981; le Manse, 1953, Hölldobler and Wilson, 1990:167), so observation of worker egg laying is not proof of male production by workers. To some extent, the phenomenon occurs (Bourke, 1988a; Choe, 1988; Hölldobler and Wilson, 1990:190); indeed in some species (mostly primitive ponerines) individuals of worker morphology not thought to be derived from queens are inseminated and lay eggs of both sexes (Lenoir and Cagniant, 1986; Peeters and Crewe, 1984). Ratnieks (1988) has suggested that workers may be selected to police against egg laying by other workers if queens are multiply mated.

If individuals can contribute directly to their fitness by laying reproductive (male) eggs, they might be expected to avoid tasks outside the nest. This would occur since tasks such as foraging are relatively dangerous; individuals may suffer predation and stand some chance of being unable to navigate return to the nest. Hence conducting tasks outside the nest is likely to reduce life expectancy. Laying workers would be expected to avoid reductions in life expectancy, and remain in locations where egg laying is possible, for the period they are fertile.

Whatever the life expectancy and reproductive status of individuals, they can be expected to work at an optimal rate if maximizing inclusive fitness. That is, we would expect individuals to maximize the value of (benefit-cost), where the benefit of activity accrues from increased survival of brood, but activity has an energetic cost, depleting colony energy level and possibly individual life expectancy.

For simplicity, let us consider that two tasks are neccessary for maintenance and growth of the colony; brood care and foraging. Hence individuals may do one of three things: tend brood, forage, or remain inactive. Individuals might operate the following algorithm to enable a choice of tasks to be made:

- tend brood, if there are brood available for tending, otherwise:
- be inactive, unless foraging is required, i.e., join the foraging pool.

Thus the preferred task of any individual is to tend brood. If that is not possible, individuals join the foraging pool, and will forage if food is required. Individuals will conserve energy (rest) if brood tending and foraging are not required. Hence these simple rules encode the principles of inclusive fitness maximization and activity level optimization discussed above. They may also allow worker allocation in appropriate proportion to the level of requirement of various tasks. This model is presented only as a verbal argument, to allow consideration of the decisions individuals may have to make in order to allocate their resources appropriately. It is similar to the more detailed model developed by Tofts (1991), discussed in Section 2.1.3. Tofts' ants make decisions as to task on the basis of shortfall and surplus of neighbouring tasks that are arranged logically as a linear sequence.

The verbal model given here clearly begs the question of how individuals assess whether brood require tending, and whether foraging is required. In both cases, synchrony may allow information to be obtained, or improve the quality of that information. Firstly, synchrony may enable workers to determine by inspection whether brood require tending, through the mechanism of mutual exclusion proposed in Section 8.3.4. If individuals are capable of laying viable reproductive eggs, we may further consider that they actually "compete" for positions on the brood pile, and that under such conditions a "gene for synchrony" might spread.

Consider an allele E (early riser) that leads to the possessor becoming active as soon as another individual becomes active. On becoming active, all individuals search for an item of brood to tend; if none are available (they are all occupied), they are forced into the foraging pool decision domain. Individuals with "early riser" phenotypes are more likely to remain on the brood pile than "late risers", those that do not respond as quickly to the activity of others. This will result as late risers are more likely to discover that all the brood are already occupied in any tending bout. Early risers will achieve a higher life expectancy, on average, than late risers, by virtue of being less likely to forage. Thus, given that workers obey the algorithm above, the allele E is likely to spread in the population as a result of early risers producing more sons than late risers. In a similar vein, Schmid-Hempel (1990) suggests that synchronous activity might be the outcome of individuals playing a workload strategy *work only when others work*, which he considers to be uncheatable.

When direct reproduction through laying of male eggs is not an option, being in synchrony may still be advantageous to individuals via maximizing the kin component of inclusive fitness. If colonies are able to produce more sexuals as a result of efficient decision making by individuals owing to synchrony, alleles that

are the ultimate basis of such behaviours will spread.

Clearly, it is in the interests of individuals, and of the colony as a whole, that "correct" decisions are made. Here "correctness" implies that individuals do not work unneccessarily, but do work when it is required. In the case of foraging, synchronized activity may allow improved information reliability (Section 8.3.2). If decisions are based on more reliable information, less colony resources may be wasted. Individuals will be less likely to undertake risky foraging operations when not required, without subjecting the colony to the risk of starvation.

Hence the appropriate features of temporal polyethism, namely that older workers forage, and younger workers tend brood, may arise as a consequence of the number of decisions that an individual has made at a given time in adult life. Temporal polyethism may therefore be an emergent phenomenon of simple rules operated by individuals to maximize their inclusive fitness. It is not neccessary to invoke age related thresholds of response to various tasks (e.g., Calabi, 1988), in order to account for temporal polyethism (Tofts, 1991), although these features may exist. Similarly, it is not neccessary to assume that laying workers become infertile when older (West-Eberhard, 1979), to account for the direction of behavioural change, although again, age related sterility may occur. Neither is it neccessary to assume that workers "know" their age, such that they take up foraging when older (Porter and Jorgensen, 1981).

8.3.7 Genetic and developmental components of behaviour

Although Tofts' (1991) model may be the simplest that can account for behavioural patterns such as temporal polyethism, it is not neccessarily correct.

There is some evidence that genetic differences between individuals are related to behavioural differences within colonies of bees (reviewed in Page and Robinson, 1991; Robinson, 1992; Page et al., 1992). There is also evidence that age related thresholds of response to stimuli change as a result of changes in Juvenile Hormone (JH) titres of adult bees (Robinson, 1992). For instance, JH titres have been shown to increase as bees age (Fluri et al., 1982), and treatment with JH, its analogs and mimics appears to induce foraging (Jaycox, 1976; Robinson 1985, 1987a, 1987b). JH levels are also associated with physiological changes (for example Rutz et al., 1976). Robinson et al. (1989) manipulated colonies to induce premature foraging and nursing by older individuals. They observed corresponding changes in JH titres, an increase in JH of the foragers and a decrease in the nurses. Hence it is not clear whether change in JH levels causes behavioural changes, or *vice versa.* As yet, there is little evidence linking JH levels to polyethism in ants (Robinson, 1992), although it has been linked to the control of physical polyethism in *Pheidole* (Wheeler and Nijhout, 1981, 1983, 1984).

It is certainly possible that in some cases behavioural change is linked to age related change in the operation of genes and their products. Hence models such as Calabi's (1988) proposing age related changes in response thresholds to stimuli, may represent the true picture for some species, even though such mechanisms may be less flexible to colony needs over the short term than simpler models (Tofts, 1991).

There seems to be no general solution to the problem of applying Occam's Razor in such cases. What may be mathematically parsimonious, in that it requires the least assumptions about organisms and the simplest mechanism to achieve appropriate ends, may not be parsimonious to biologists considering evolutionary

routes to achieving those properties. Numerous examples are quoted in the literature of evolutionary biology (for example, Gould and Lewontin, 1979). Social insects have evolved from solitary species, in which the ontogeny of behaviour has genetic and hormonal determinants. Given the existence of such mechanisms in evolutionary ancestors, they may have been retained in social insects. Alternatively, they may have been displaced by other mechanisms that function as a result of social interactions and colony context. It is possible that a variety of hormone and interaction based mechanisms exist, that are used to varying extents in different species, depending on features of colony size and physical structure, and environmental variability.

8.3.8 Conclusion

In common with other research, I have demonstrated that colonies of *L. acervorum* appear to possess activity patterns such that most individuals are active, or inactive, simultaneously. However, I have been unable to demonstrate true periodicity in that the period is not fixed or narrowly and normally varying about a mean. The activity cycles appear to be remarkably robust; cycle time being independent of colony size, brood to worker ratio or the energy level of the colony (manipulated by starvation). Cycles appear to occur as a result of two properties of individuals:

1. they are stimulated to activity by physical contact with others;
2. stimulation generally does not result in response for some lag period in the order of 10 minutes after an individual becomes inactive.

These properties lead to positive feedback in the activation phase of the cycle, such that individuals tend to synchronize their active phases.

I have discussed a number of possible advantages for synchronized activity as opposed to random activity patterns leading to asynchrony in the activity of individuals. These advantages mainly concern improved reliability in information exchange, leading to improved colony efficiency in task performance and task allocation. Although cyclical activity is a holistic phenomenon, observable only at the colony level, this does not entail selective explanation at the group level. Individuals that compose the colony are advantaged by increased production of kin as a result of improved efficiency of colony operations. Hence colony level phenomena may have mechanistic and evolutionary explanations at the individual level.

If synchrony is advantageous, one may ask why synchrony is not more complete. The data I present clearly show that not all individuals synchronize at every activity bout, leading to "messy" cycles at the colony level. Several possibilities may be of relevance here. Firstly, the cost of maintaining more accurate mechanisms allowing perfect synchrony may outweigh the benefits accrued. Secondly, perfect synchronization may be disadvantageous, in that it entails complete synchronization of the inactive phase. A perfectly inactive colony may be unable to respond to immediate threats, such as invasion by predators. Therefore, it may pay colonies to maintain some active "look outs" during the inactive phase. Thirdly, complete synchrony may be impossible to achieve for a variety of reasons. For instance, the time required to complete certain tasks such as foraging may exceed the colony active phase, individuals performing that task may automatically desynchronize. Also, it may not be possible (or desirable; Pasteels et al., 1983) to select out some degree of inaccuracy in individuals.

Although we can demonstrate theoretically that synchrony may be advantageous, there is no empirical evidence as yet that synchronous activity is advantageous to social insect colonies. It is also difficult to conceive of advantages for short term periodicity in activity. The observed near periodic activity patterns might best be considered as the inevitable outcome at the colony level of basic behavioural features of individuals. Whether these features, such as probability of response to external stimuli and probability of "spontaneous" activity onset as a function of time spent inactive have been honed by selection for synchrony, remains to be investigated.

8.4 Further Work

A number of features of activity cycles and related phenomena remain to be examined:

Robustness

For *L. acervorum*, cycle length appears to be fairly robust with respect to colony size, brood to worker ratio and colony energy level. It would be interesting to determine whether cycle length varies with temperature. Circadian rhythms do not vary appreciably with temperature, although initially they may "reset" (Pittendrigh, 1981a). Lack of variation with temperature change may indicate that the internal clocks of individuals (underlying the duration of the inactive phase) operate by mechanisms similar to those underlying circadian clocks, whereas cycle length variation with temperature may indicate that response probabilities of individuals can vary with physical (environmental) factors. Similarly, the effect

of light intensity and seasonality on cycle length has yet to be examined.

Between species comparisons

Certain arguments concerning the advantages of synchrony might be open to investigation using comparative techniques. The data presented in this thesis, and in the papers of Cole (1991a,b,c) suggest that activity cycle length may vary between species within the genus *Leptothorax*. Cycle length, and propensity of individuals to synchronize might be related to features such as degree of polygyny, rate of brood development and production, and frequency of worker male egg laying, or species tempo (Oster and Wilson, 1978:281). Leptothoracines provide valuable research tools for investigating these possibilities; the genus comprises a variety of species ranging in colony growth rate, degree of polygyny and frequency of worker egg laying (e.g., Buschinger, 1968; Bourke, 1988a, 1991; Heinze and Buschinger, 1988). Social parasitism in the form of inquilinism and dulosis (Bourke and Franks, 1991; Buschinger, 1990) also enables us to investigate whether parasitized colonies behave in similar ways. The small size of mature colonies and ease of laboratory maintenance so that the whole colony can be observed also makes them suitable research subjects.

It remains to be discovered whether cyclicity and synchrony is widespread in other social insects. Comparison with more distantly related species may enable us to determine whether synchrony is a primitive or derived trait, and whether the more complex spatial structure of nests or behavioural organizations such as physical polyethism preclude its occurrence.

Information

Simultaneous observations of many workers and brood may allow us to determine whether mutual exclusion is indeed occurring on the brood pile, and to what extent it may improve task allocation and reduce task repetition. As discussed in Section 8.3.4, these studies combined with studies on the effect of neglect on brood survival could indicate whether synchrony could yield an adaptive advantage via passive information exchange. It is more difficult to conceive of tests for the advantage of synchrony to direct information exchange, for instance in determining foraging decisions. Perhaps cross species or population comparative studies of synchrony in relation to foraging risk would be illuminating.

Response to starvation

In Chapter 6, I introduce some interesting features of colony response to the stress of starvation from the within nest perspective. The effect of food deprivation on the interactions of individuals and their propensity towards cyclical activity deserves further attention. Observations of individual behaviour, combined with colony level measurement approaches may enable us to link intranidal responses to what is known about extranidal responses, and to interesting developments in the understanding of honeybee foraging organization (Seeley, 1989a, 1989b; Seeley et al., 1991), and self organization approaches to social insect behaviour (see Chapter 1).

Spatial structure

In Chapter 7 I introduce a simple automated technique for measuring some features of colony organization in space. At present it is not clear what features are being measured. The pattern of correlation between activity levels in different regions of the nest appears to be strongly dependent on the physical structure of the nest, however patterns appear to emerge from apparently homogenous, single chambered nests also. The noninvasive and objective nature of this technique would argue for its further investigation. Measurments of colonies inhabiting nests of different shape, or subjected to manipulations of brood to worker ratio or food deprivation may be illuminating.

Signal propagation

In parallel with the preceeding suggestion, the rate of signal propagation and its point(s) of origin within the nest have yet to be measured. Image analysis techniques that allow frames to be grabbed at shorter time intervals would be useful here. If signal propagation can be more accurately measured it might be possible to determine whether the assumptions of Tofts' (1990) model are appropriate. It may also be possible to determine whether activity on the brood pile or at the nest entrance drives the colony cycle, by considering points of signal initiation. This may enable us to test hypotheses concerning the function or advantages of synchrony.

Measurement of individuals

Although there is clearly scope for extension of image analysis techniques in this field, further direct observation of individuals is required if we are to link automated measurments to actual behaviour. In the narrow context of this thesis, observations are still required to determine response probabilities as a function of time spent in the inactive phase. Such observations are required if we wish to test Tofts' (1990a) latter model of autosynchronization.

More broadly, although automated techniques can provide large quantities of data, the quality is low: we can measure activity, but we cannot say what that activity represents, apart from movement. Approaches that link image analysis to direct observation, for instance by considering activity and the spatial location of tasks within the nest would be most interesting.

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Appendix A

Methods

Table A.0.1

	Innen	*Aussen*	*Row Totals*		*Row No.*
			T_r	$T_r lnT_r$	
Column No.	*1*	*2*	*3*	*4*	
Re	*266.169*	*80.472*	*89*	*399.488*	*1*
IM	*626.916*	*586.091*	*251*	*1386.88*	*2*
GS	*235.506*	*372.189*	*142*	*703.727*	*3*
BC	*271.335*	*29.819*	*77*	*334.473*	*4*
GBW	*10.751*	*23.026*	*16*	*44.361*	*5*
GW	*10.751*	*26.377*	*17*	*48.165*	*6*
ExF	*3.296*	*55.944*	*22*	*271.335*	*7*
IACW	*36.947*	*200.523*	*65*	*271.335*	*8*
RACW	*40.621*	*142.879*	*54*	*215.405*	*9*
Column Totals	T_c *360* $T_c lnT_c$ *2118.997*	*373* *2208.748*	*Grand Total N 733* $d = NlnN$ *=4835.7*		*10* *11*

FlnF transforms of all observed frequencies (F) in Table 2.2.4. T_r *is the total of observed frequencies in given row.* T_c *total of observed frequencies in given column. Innen, Aussen refer to two task groups as defined in Chapter 2. Behavioural act abbreviations (Re...RACW) are identified in Tables A.0.1 and 2.6.*

Table A.0.2 *Calculation of G statistic values and formulae.*

G statistic	*Value*	*df*	*Calculation*
G_H *(heterogeneity)*	*111.46**	*8*	$2(a-b-c+d)$
G_P *(pooled)*	*5.22**	*1*	$2(360ln\frac{360n}{16}+373ln\frac{373n}{14}-d)$
$G_I(R)$ *(individual)*	*See Table 2.2.6*	*1*	$2(e-f-g)$
G_T *(total)*	*116.68**	*9*	G_H+G_P *or* $\sum_{R=1}^{R=9}G_I(R)$

Values marked (*) indicate significant ($P < 0.05$) *departure from null hypothesis:* ($\chi^2_{0.05,1df} = 3.8$, $\chi^2_{0.05,8df} = 15.5$). *Null hypothesis:* G_H, *no association between frequency of act performance and task group;* G_P, *no association between total number of acts performed and task group. The parameters used above are defined as follows:*

calculation of G_H*:*

$$a = \sum FlnF$$

for all frequencies. (Sum of values in columns 1 and 2, rows 1 to 9 of Table A.0.1 = 3019.612)

$$b = \sum T_r lnT_r$$

for row sums. (Sum of values in column 4, rows 1 to 9 of Table A.0.1= 3471.837)

$$c = \sum T_c lnT_c$$

for column sums. (Sum of values in row 11, columns 1 and 2 Table A.0.1=4327.745)

$d = NlnN$

where N is the grand total of acts observed ($d = 4835.7$).

Calculation of G_P:

n the number of observed individuals (=30). d as given for G_H.

Calculation of $G_I(R)$:

as given above, for given row R.

$e = Fln\frac{F}{\hat{P}}$; *F value for row R column 1 (innen);*

$f = Fln\frac{F}{\hat{P}}$; *F value for row R column 2 (aussen);*

$g = T_r lnT_r$; *T_r for sum of row R;*

$\hat{P}$ is the expected proportion of total acts (in given row) performed by a given group (column). In all cases, $\hat{P} = \frac{1}{2}$, that is, equal probability of performance of any act by Innen or Aussen. Method from Sokal and Rohlf (1981:722) also Fagen and Young (1978).

Table A.0.3

	Re	*IM*	*GS*	*BC*	*ExF*	*IACW*	*RACW*	*FdW*	*MO*	T_R *Tot*
Re	*0*	*26.44*	*15.94*	*9.14*	*2.59*	*7.91*	*6.54*	*4.20*	*11.24*	*84*
IM	*56.21*	*0*	*52.49*	*30.11*	*8.55*	*26.04*	*21.57*	*0*	*37.03*	*212*
GS	*18.08*	*43.47*	*0*	*15.03*	*4.27*	*13*	*10.77*	*6.91*	*18.48*	*130*
BC	*10.23*	*24.59*	*14.82*	*8.50*	*2.41*	*7.35*	*6.09*	*0*	*0*	*74*
ExF	*2.43*	*5.84*	*3.52*	*2.02*	*0.57*	*1.75*	*1.45*	*0.93*	*2.49*	*21*
IACW	*7.52*	*18.09*	*10.90*	*6.25*	*1.78*	*5.41*	*4.48*	*2.87*	*7.69*	*65*
RACW	*6.25*	*15.03*	*9.06*	*5.20*	*1.47*	*4.49*	*3.72*	*2.39*	*6.39*	*54*
FdW	*6.77*	*0*	*9.81*	*0*	*1.60*	*4.87*	*4.03*	*0*	*6.92*	*34*
MO	*14.00*	*33.66*	*20.29*	*0*	*3.30*	*10.07*	*8.33*	*5.35*	*0*	*95*
T_C *Tot*	*89*	*214*	*129*	*74*	*21*	*64*	*53*	*34*	*91*	*769* $=N$

Null hypothesis for single step transitions between behavioural acts for all ants. Row, column and grand total data are those presented in Table 2.2.8. Single step transitions that cannot occur as a result of the definition of the end of an act , as in some self transitions,(eg Re-Re, or due to spatial incompatibility of acts such as BC-FdW) are given expected frequency 0.

Calculation of nonzero frequencies expected frequency (f) of given transition (R_i, C_j) *(1st act row and second act column):*

$$f(C_i, R_j) = \frac{T_r \times T_c}{N - \sum_X T_{i,X}}$$

Where N *is the total number of observed acts (769);* $T_{i,X}$ *are the column totals for all transitions that cannot occur between the given R (first act) and following act* X*. For example*

$$f(FdW, RE) = \frac{34 \times 89}{769 - 214 - 74 - 34} = 6.77$$

Explanation: If no transitions are restricted (i.e., all possible transitions can

occur) the expected frequency for a given transition is simply:

$$f(C_i, R_j) = \frac{T_{R_j} \times T_{C_i}}{N}$$

(Sokal and Rohlf, 1981:733; model 1 no fixed marginals). If some transitions are restricted (cannot occur) the expected frequency for a given transition $f(R_i, C_j) = 0$, *or*

$$f(R_i, C_j) = \frac{T_{C_j} \times T_{R_i}}{\textit{Total No. of acts observable after } R_i}$$

The value of the denominator is given by; total number of acts - total number that cannot occur after R_i.

Transitions were analysed by χ^2; χ^2 *was calculated by:*

$$\chi^2 = \frac{(O-E)^2}{E}$$

the expected values given as above; observed values in Table 2.2.8.

Individual transitions were judged to be associated (positive or negative) if the cell value of χ^2 *exceeded* $\chi^2_{0.05,1df} = 3.8$. *By summing all cell values a* χ^2 *value for overall heterogeneity of transitions was obtained (See Table 2.2.8). The summed value was judged against* $\chi^2_{0.05,55df}$, *degrees of freedom calculated as follows*

$$df = \sum_{i=1}^{i=(q-1)}((\textit{no. cells in row } i) - 1)$$

where q is the number of rows.

Table A.0.4

	Re	*IM*	*GS*	*BC*	*IACW*	*RACW*	*Totals* (T_R)
Re	*0*	*23.56*	*11.26*	*12.93*	*2.71*	*3.54*	*54*
IM	*31.32*	*0*	*31.32*	*35.96*	*7.54*	*9.86*	*116*
GS	*11.05*	*23.12*	*0*	*12.69*	*2.6*	*3.48*	*53*
BC	*10.87*	*22.74*	*16.87*	*12.48*	*2.62*	*3.42*	*63*
IACW	*1.90*	*3.97*	*1.90*	*2.18*	*0.45*	*0.60*	*11*
RACW	*2.76*	*5.78*	*2.76*	*3.17*	*0.67*	*0.87*	*16*
Totals (T_C)	*54*	*113*	*54*	*62*	*13*	*17*	*313 (=N)*

Null hypothesis (expected frequencies) for single step transitions between behavioural acts for Innen task group. For explanation of value calculations see Table A.0.3.

Table A.0.5

	Re	*IM*	*GS*	*IACW*	*RACW*	*FdW*	*MO*	*Totals* (T_R)
Re	*0*	*6.73*	*6.07*	*4.14*	*2.74*	*2.83*	*7.48*	*30*
IM	*10.81*	*0*	*23.20*	*15.89*	*10.49*	*0*	*28.61*	*89*
GS	*8.02*	*19.12*	*0*	*11.80*	*7.79*	*8.02*	*21.24*	*76*
IACW	*4.05*	*9.64*	*8.69*	*5.95*	*3.93*	*4.05*	*10.71*	*47*
RACW	*2.07*	*4.92*	*3.04*	*2.01*	*2.07*	*2.07*	*5.47*	*24*
FdW	*4.13*	*0*	*8.86*	*6.07*	*4.01*	*0*	*10.93*	*34*
MO	*10.59*	*25.23*	*22.74*	*15.57*	*10.28*	*10.59*	*0*	*95*
Totals (T_C)	*34*	*81*	*73*	*50*	*33*	*34*	*90*	*395*

Null hypothesis (expected frequencies) for single step transitions between behavioural acts for Aussen task group. For explanation of value calculations see Table A.0.3.

Table A.0.6

n	*t*	*95% Confidence Intervals*	
		Lower	*Upper*
392	*260*	*261.67*	*268.33*
393	*260.67*	*252.33*	*269.01*
394	*261.33*	*252.98*	*269.68*
395	*262*	*253.64*	*270.36*
396	*262.67*	*254.30*	*271.04*
397	*263.33*	*254.95*	*271.72*
398	*264*	*255.61*	*272.39*
399	*264.67*	*256.26*	*273.07*
400	*265.33*	*256.92*	*273.75*
401	*266*	*257.58*	*274.42*
402	*266.67*	*258.23*	*275.10*
403	*267.33*	*258.89*	*275.78*
404	*268*	*259.54*	*276.46*
405	*268.67*	*260.20*	*277.13*
406	*269.33*	*260.86*	*277.81*
407	*270*	*261.51*	*278.49*
408	*270.67*	*262.17*	*279.16*
409	*271.33*	*263.48*	*279.84*
410	*272*	*263.48*	*280.52*
411	*272.67*	*264.14*	*281.20*
412	*273.33*	*264.79*	*281.87*
413	*274*	*265.45*	*282.55*
414	*274.67*	*266.11*	*283.22*
415	*275.33*	*266.76*	*283.90*

95% confidence intervals of Kendall's (1976) turning point statistic for relevant numbers of independent observations. The numbers of independent observations (n) given are those relevant for comparison of the activity time series presented in Chapters 4, 6, 7. t refers to the expected number of turning points in a random series of length n.

Notes on calculation. n is the number of independent observations (discounting 1 observation per pair if consecutive observations have the same value); hence n is the length of the time series minus the number of paired observations.

t is the expected number of turning points in a random series of length n:

$$t = \frac{2}{3}(n-2)$$

confidence intervals for 95% confidence, the range of turning points that may be expected from a random series of length n is estimated as:

$$t +/- (\frac{(16n-29)}{90})^{\frac{1}{2}}$$

A.1 Autocorrelation

Autocorrelation was conducted as MINITAB for activity time series of 415 values of x_t.

For i=1 to 100

calculate Pearson product moment correlation between x_t and $x_{(t+i)}$

next i

Output correlation coefficient for each i

(x_t datum value for time t). The Pearson product moment coefficient r_{jk}

$$r_{jk} = \frac{\sum x_j x_k}{(n-1)s_j s_k}$$

with s_j and s_k the standard deviation of variables x_j and x_k respectively. Significance ($P < 0.05$) is judged by comparison to the critical value for r which for

$n = 415$ is

$$|r_{jk}| > r_{0.05,414df} = 0.098$$

A.2 Trough Location Procedure

Cycle length was measured from time series data as the interval (in seconds) from succesive local minima. Local minima were located in the time series using the program SPREDLOC.BAS (see Appendix C.2). Each point x_i of the time series was replotted as the minimum in a local field from $x_i - w$ to $x_i + w$, where w is the field width. A width of 4 was used for all analyses, to remove very short term fluctuations in the data, without obscuring variations on the time scale I was attempting to measure. The trough location procedure can be summarized as follows:

```
for i from 4 to n-4
   find minimum x(i) in the field i-4 to i+4 (min(i))
next i
for i from 4 to n-4
  plot min(i)
next i
locate minima by eye
mark positions of succesive troughs using mouse
calculate inter trough interval and store
output intervals as measures of cycle length
```

A.3 Calculation of third and fourth moments

Third and fourth moments for the cycle length data (amalgamated over all days for a given run; cycle length measures from trough location, Appendix A.2) were calculated as given by Sokal and Rohlf (1981:114):

Third moment $g_1 = \frac{1}{ns^3}\sum(X - \overline{X})^3$

Fourth moment $g_2 = (\frac{1}{ns^4}\sum(X - \overline{X})^4) - 3$

Where n is the sample size, s is the sample standard deviation, X the datum, and $\overline{X}$ the mean of the data.

Significant deviations ($P < 0.05$) from a normal distribution were tested using the T test, incorporating the known standard errors of g_1 and g_2 (Sokal and Rohlf, 1981:139,174):

Standard error of g_1 is approximately $\sqrt{\frac{6}{n}}$

Standard error of g_2 is approximately $\sqrt{\frac{24}{n}}$

for sample sizes greater than 150.

The t-statistic is given by

$$t_s = \frac{(g-y)}{s_g}$$

Where y is a parametric value, y_1 and y_2 are 0 for a normal distribution. The appropriate degrees of freedom is infinity.

Hence g statistic deviates significantly from normal if:

$$|t_s| > 1.960$$

Siginificant positive g_1 indicates a right skewed distribution; significant positive g_2 indicates leptokurtosis.

A.4 Calculation of Expected Distribution

Tofts (1990a) predicts that cycle length will be distributed as a geometric decay initiated at a lag s. The maximum likelihood distribution was calculated using known characteristics of the geometric distribution (e.g., Chatfield, 1988:186).

pdf	$p(1-p)^{r-1}$	(1.i)
mean	$\frac{1}{p}$	(1.ii)
variance	$\frac{(1-p)}{p^2}$	(1.iii)

(where p = probability of success, r takes the values 1,2,.... and is equivalent to time step).

However, the distribution predicted by Tofts (1990a) incorporates the geometric distribution after a period s (equivalent to the "base sleep time" of individuals):

pdf	0 for $r < s$	
pdf	$p(1-p)^{r-1}$ for $r \geq s$	(2.i)
mean	$\frac{1}{p} + s$	(2.ii)
variance	$\frac{(1-p)}{p^2}$	(2.iii)

Hence, it is neccessary to estimate s in order to generate the required distribution. s is estimated as follows:

1. calculate the value of the mean (m_{data}) and variance (v_{data}) of the data;
2. calculate the value of p (p_{calc}) by substituting v_{data} into equation 1.iii or 2.iii: $v = \frac{(1-p)}{p^2}$ with solutions in the range $0 < p < 1$ $p = \frac{((2+\frac{1}{v})-(\sqrt{(2+\frac{1}{v})^2-4}))}{2}$
3. calculate s by substituting m_{data} and p_calc in equation 2.ii.

The required geometric distribution is generated as follows:

1. locate the maximum value of cycle length from the data ($maxv$);
2. subtract s from $maxv$ ($max1$);
3. partition the total interval 0 to $max1$ into 10 equal intervals;
4. calculate the expected number of values (Exp) that fall within each interval:

$$Exp(r, r') = N \times \Sigma_{i=r}^{i=r+r'} pdf(i)$$

where N = total no. of observations (from the data); r' = time steps (in seconds) that lie within a given interval, starting at r

Hence, the expected number of successes (starts of colony activity bouts) at each time step is calculated by multiplying the probability of success at that step by

the total number of observations over all steps. The expected number of successes within each interval is calculated by summing all the expected successes for time steps that lie within that interval.

Upper and lower limits to p and s were calculated by substitution into the above equations, with the mean and variance set at the bounds of the error range.

The limits to the mean are given by

$$mean_{max/min} = mean_{data} + / - 1.96\sqrt{\frac{\sigma^2}{n}}$$

σ^2 is the variance; n is the sample size. Similarly the limits of the variance:

$$v_{max/min} = v_{data} + / - 1.96\sqrt{\frac{v^2_{data}}{2n}}$$

Which gives limits to p and s respectively of:

$$p_{max} = \frac{1}{2}(2 + \frac{1}{v_{min}} - (\sqrt{(2 + \frac{1}{v_{max}})^2 - 4}))$$

$$p_{min} = \frac{1}{2}(2 + \frac{1}{v_{max}} - (\sqrt{(2 + \frac{1}{v_{min}})^2 - 4}))$$

$$s_{max} = mean_{max} - \frac{1}{1-p_{min}}$$

$$s_{min} = mean_{min} - \frac{1}{1-p_{max}}$$

Nine test distributions were calculated from the combination of the maximum likelihood estimates for p and s together with the upper and lower limits of each parameter. (see Figure A.1)

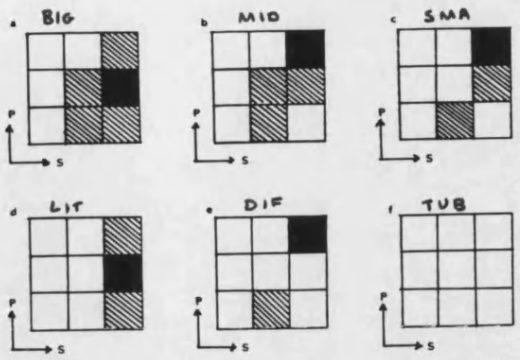


Figure A.1: Test distribution fitting the data in Chapter 4. For each run, test distributions that did not depart significantly from the data are shown by shaded boxes corresponding to the appropriate estimates for s and p. Best fit distribution is shown in black.

Goodness of fit of the observed distribution to each test distribution was calculated using X^2 over 10 intervals (the last 3 being summed to ensure expectations greater than 5) resulting in 7 intervals actually being used. Fit was decided using X^2 value to $X^2_{0.05,5df} = 11.07$. For further details of test procedure see Chapter 4.

A.5 Test of Trough Location Procedure

Blind tests were conducted in order to assess the importance of subjective components in the trough location procedure (Appendix A.2) used to measure cycle length. From the data presented in Chapter 6, it was noted that cycle length appeared to decrease under prolonged food deprivation, although not significantly. This nevertheless represented the clearest difference in cycle length between the runs for *L. acervorum*. Blind comparison of cycle lengths between species seemed inappropriate as the time series varied obviously in form. Hence a blind test was conducted on the data from the run STA.

Blind Test

The time series of the first seven days (STA01-STA07) and the last seven days of starvation (STA23-STA29) were relabelled (T1-T14) By T. Stickland in a random order. Each series was presented in order of the new label using TSPRED.BAS. This program was identical to SPREDLOC.BAS, except that the vertical axis (number of pixel mismatches) was not labelled. and the height representing unit pixel change was kept constant; with the maximum pixel change plotted at a fixed height on the screen. These precautions were taken so that overall activity level and amplitude would not allow identification of files (since series at the start of the run show higher levels of activity). I then located troughs in the usual manner (see Appendix A.2) for each series. This process was repeated to obtain 3 sets of cycle length measurements without prior knowledge of the identity of any series. The labels were then decoded to obtain 3 sets of cycle length statistics for starved and fed days, as analysed by the program ASTATA.BAS (see Appendix C.1). The results were compared to 3 sets measured by myself when I was aware of the series identities (see Table A.5.1).

The results suggest no obvious difference between batches formed from identified or non-identified files. Both sets of batches from fed ants (KF, RF) exhibit a large cycle length compared to starved ants (KS, RS), although the difference is not significant (T test $P < 0.05$). The variances of each batch do not appear to differ consistently between batches of K or R, nor do the values of X^2 for goodness of fit to the maximum likelihood distribution, or the counts of test distributions (out of 9) that fit the data ($P > 0.05$). Hence it appears that even slight differences in cycle length can be recorded consistently using the trough location procedure when the identity of the file is not known in advance, and precautions are taken to obscure aspects of the time series that might allow their identification.

Table A.5.1

Batch	*n*	*Mean*	*Var*	*MLE*	*Number* < 11.07
KE:1	*118*	*1312*	*116714*	*26.01*	*0*
KE:2	*115*	*1326*	*106527*	*11.02*	*2*
KE:3	*119*	*1290*	*120131*	*8.50*	*3*
KS:1	*124*	*1245*	*138213*	*9.89*	*2*
KS:2	*126*	*1233*	*134140*	*15.43*	*0*
KS:3	*123*	*1268*	*131065*	*10.21*	*3*
RF:1	*114*	*1344*	*102369*	*40*	*3*
RF:2	*117*	*1322*	*115731*	*28.9*	*0*
RF:3	*116*	*1336*	*108287*	*12.04*	*1*
RS:1	*132*	*1231*	*131024*	*17.85*	*0*
RS:2	*123*	*1272*	*148814*	*10.95*	*2*
RS:3	*126*	*1248*	*138523*	*8.86*	*2*

3 sets of cycle length statistics for the first seven days (batches KF, RF) and the last seven days (batches KS, RS) of the run STA are presented; batches prefixed K indicate that the file identity was known; batches prefixed R indicate file identity was unknown and the files were presented in random order. n, the number of cycles measured; mean, the sample mean (in seconds); Var the variance of the sample; MLE value of X^2 *goodness of fit for the maximum likelihood distribution; Number* < 11.07 *is the number of test distributions that fit the data* ($P \geq 0.05$).

A.6 Estimation of Correlation Frequency

An estimate of the probability of obtaining a significant correlation (where $r_{obs} > R_{crit}; P < 0.05$) between independent cyclical data sets was made by performing cross correlations between time series of the same run on different days:

procedure for empirical estimation of significant correlations resulting from random effects:

For each run:

1. choose 2 run dates at random
2. calculate correlation coefficients for all pairs of timeseries between different windows on different dates. The windows considered are all those which will be cross correlated for a single day as appropriate to the experiment
3. score all significant coefficients:

$$S+ = No(r_{obs} > r_{crit})$$

$$S- = No(r_{obs} < -r_{crit}) \ldots . r_{crit;0.05,(n-1)df}$$

4. repeat for the other pairs of run dates chosen at random from within a given run
5. calculate the probability of obtaining a significant correlation from independent data sets:

$$PS+ = P(\text{ obtaining } r_{obs} > r_{crit}):$$

$$PS+ = \frac{S+}{\text{total pairwise correlations performed}}$$

the total number of pairwise correlations $= (N-1)^2 \times R$ where $N =$ no. windows; $R =$ no. repeats at stage 4. Correlations were performed using MINITAB.

The results are presented in Table A.6.1. By multiplying the value of p_r (probability of significant correlation from random pairing of series) by k (the number of trials; in the case of 1POK=16), a threshold score s_t of correlations is obtained; a pair of series are judged to correlate positively if the observed number of correlations s_o exceeds the upper limit of the 95% confidence interval of s_t. Confidence

intervals are set on the basis of the known standard deviation (s_r) of the binomial distribution (p is the probability of success, i.e., finding a correlation). For example, 1POK correlations:

$$p_r = p = 0.0814$$

with 16 trails $\mu = kp = 1.3024$ and $\sigma = \sqrt{kp(1-p)} = 1.094$. So the lower confidence interval is $\mu - 1.96\sigma = -0.842$, and the upper is $\mu + 1.96\sigma = 3.45$, which as the distribution is discrete gives us limits of 0 and 4 respectively.

Table A.6.1

Run	*n*	p_r *+ve*	s_t^+ *(N)*	*LCI*	*UCI*
1POK	*676*	*0.0814*	*1.302 (16)*	*0*	*4*
GRID	*507*	*0.0769*	*0.615 (8)*	*0*	*3*
BOX	*845*	*0.0805*	*0.805 (10)*	*0*	*3*
TUB	*676*	*0.170*	*2.72 (16)*	*0*	*6*
Run	*n*	p_r *-ve*	s_t^- *(N)*	*LCI*	*UCI*
1POK	*676*	*0.0725*	*1.16 (16)*	*0*	*4*
GRID	*507*	*0.1085*	*0.868 (8)*	*0*	*3*
BOX	*845*	*0.0769*	*0.769 (10)*	*0*	*3*
TUB	*676*	*0.046*	*0.736*	*0*	*3*

Estimation of probability of obtaining significant correlations from series paired at random. Estimates were made for each run, by scoring the number of significant positive (p_r +ve) and negative (p_r -ve) correlations found in a large sample (n) of paired series from different run days. s_t (N): estimated mean number of significant correlations over N trials. For calculations of means and confidence estimates see text.

Appendix B

Tables

Table B.0.2

Co-occurrence of behaviour in 30 minute samples

	T	*Re*	*IM*	*GS*	*BC*	*GBW*	*GW*	*ExF*	*IA*	*CNM*	*RA*	*FdW*	*MO*
Re	24												
IM	29	24											
GS	27	22	26										
GBW	10	7	10	8	4								
GW	9	7	9	9	6	2							
ExF	11	10	11	10	3	4	3						
IA	18	14	18	16	6	4	7	10					
CNM	4	3	4	4	3	3	2	3	3				
RA	24	17	23	22	10	8	7	10	17	4			
FdW	11	6	10	9	1	4	3	6	9	2	11		
MO	14	8	13	13	2	5	5	8	12	3	14	11	
RO	6	3	5	6	0	0	2	2	5	0	6	6	6

Scores refer to the number of ants (max=30) observed to perform both behaviours in a sampling period. Totals (first column) indicate the number of ants observed to perform a given behaviour in the sampling period. χ^2 *values for individual*

cells revealed no significant departure from the null hypothesis of no association ($P > 0.05; \chi^2_{0.05,1df} = 3.8$) *summed* χ^2 *values indicate no overall heterogeneity* ($P > 0.05; X^2_{0.05,60df} = 79.1$). *Expected frequencies calculated thus* $f(i,j) = \frac{T_i \times T_j}{30}$ *where* T_i *and* T_j *are the total number of ants observed performing behaviours* i *and* j *respectively.*

Table B.0.3

Turning points in daily time series from Chapter 4

Run	*Day*	*n*	*TPs (obs)*
BIGRUN	*03*	*413*	*211**
	04	*411*	*203**
	05	*415*	*179**
	06	*411*	*205**
	07	*411*	*192**
	08	*410*	*202**
	09	*410*	*202**
	10	*408*	*170**
	11	*409*	*169**
	12	*410*	*160**
	13	*413*	*175**
	14	*411*	*175**
	15	*407*	*179**
	16	*412*	*147**
	17	*412*	*165**
	18	*410*	*176**

Run	*Day*	*n*	*TPs (obs)*
MIDRUN	*01*	*411*	*200**
	02	*412*	*220**
	03	*410*	*210**
	04	*407*	*202**
	05	*408*	*198**
	06	*410*	*223**
	07	*407*	*198**
	08	*407*	*199**
	09	*410*	*223**
	10	*411*	*208**
	11	*404*	*206**
	12	*409*	*195**
	13	*408*	*220**
	14	*413*	*249**
	15	*411*	*216**
	16	*415*	*237**

Run	Day	n	Tps (obs)
LITRUN	01	405	202*
	02	406	214*
	03	406	192*
	04	410	222*
	05	413	207*
	06	409	206*
	07	410	231*
	08	405	198*
	09	403	218*
	10	408	218*
	11	411	219*
	12	407	187*
	13	407	209*
	14	407	173*
	15	406	202*
	16	409	212*

Run	Day	n	TPs (obs)
SMARUN	01	410	226*
	02	405	212*
	03	399	200*
	04	402	211*
	05	399	234*
	06	405	231*
	07	394	208*
	08	399	215*
	09	403	214*
	10	399	203*
	11	400	194*
	12	395	212*
	13	402	221*
	14	404	220*
	15	403	211*

Run	Day	n	Tps(obs)
DIFRUN	01	404	211*
	02	409	202*
	03	408	212*
	04	403	232*
	05	393	211*
	06	401	232*
	07	396	212*
	08	392	217*
	09	400	216*
	10	397	208*
	11	2396	234*
	12	402	198*
	13	403	227*
	14	403	209*
	15	395	219*
	16	398	205*
	17	297	213*

Run	Day	n	TPs (obs)
TUBRUN	01	404	199*
	02	410	197*
	03	405	194*
	04	405	195*
	05	402	179*
	06	404	208*
	07	406	198*
	10	406	207*
	11	404	202*
	12	407	191*
	13	406	207*
	14	405	199*
	15	410	198*
	16	408	195*
	17	397	199*
	19	403	198*

Results are presented for each day of each run; in each case the time series is that of activity level in window X0. n is the number of independent data points, with (*) indicating a significant ($P < 0.05$) departure from random expectation. For explanation see Table A.0.6

Table B.0.4

Samples of turning points in various windows.

Run Day	*Win*	*n*	*TPs*
MIDRUN 01	*01*	*404*	*181**
	02	*406*	*195**
	03	*353*	*299+*
	04	*373*	*222**
	05	*336*	*157**
	06	*387*	*216**
	08	*384*	*218**
	09	*362*	*183**
	X1	*409*	*205**
	X2	*400*	*228**

Run Day	*Win*	*n*	*TPs*
MIDRUN 10	*01*	*408*	*184**
	02	*399*	*177**
	03	*365*	*329+*
	04	*391*	*234**
	05	*378*	*201**
	06	*390*	*223**
	08	*388*	*228**
	09	*381*	*199**
	X1	*405*	*196**
	X2	*399*	*229**

Run Day	*Win*	*n*	*TPs*
LITRUN 01	*01*	*408*	*201**
	02	*400*	*206**
	03	*210*	*75**
	04	*377*	*224**
	05	*320*	*148**
	06	*368*	*189**
	08	*393*	*232**
	09	*157*	*48**
	X1	*399*	*210**
	X2	*399*	*220**

Run Day	*Win*	*n*	*TPs*
LITRUN 10	*01*	*404*	*189**
	02	*402*	*186**
	03	*377*	*216**
	04	*386*	*215**
	05	*327*	*161**
	06	*248*	*201*
	08	*395*	*232**
	09	*218*	*71**
	X1	*402*	*201**
	X2	*398*	*215**

Run Day	*Win*	*n*	*TPs*
SMARUN 01	*01*	*408*	*232**
	02	*405*	*220**
	03	*354*	*186**
	04	*391*	*217**
	05	*330*	*157**
	06	*343*	*171**
	08	*352*	*187**
	09	*93*	*30**
	X1	*386*	*200**
	X2	*388*	*208**

Run Day	*Win*	*n*	*TPs*
SMARUN 10	*01*	*399*	*199**
	02	*391*	*206**
	03	*130*	*18**
	04	*377*	*208**
	05	*308*	*138**
	06	*317*	*146**
	08	*348*	*190**
	09	*78*	*15**
	X1	*381*	*193**
	X2	*389*	*215**

Run Day	Win	n	TPs
DIFRUN 01	01	395	193*
	02	380	200*
	03	324	237+
	04	366	204*
	05	322	150*
	06	330	158*
	08	120	21*
	09	35	6*
	X1	385	194*
	X2	373	190*

Run Day	Win	n	TPs
DIFRUN 10	01	391	187*
	02	370	173*
	03	331	239+
	04	369	200*
	05	325	144*
	06	363	189*
	08	196	112
	09	349	194*
	X1	370	192*
	X2	376	201*

Run Day	Win	n	TPs
BIGRUN 03	01	408	197*
	02	407	202*
	03	339	171*
	04	371	180*
	05	363	191*
	06	311	146*
	08	393	221*
	09	229	69*
	X1	402	203*
	X2	386	194*

Run Day	Win	n	TPs
BIGRUN 12	01	411	154*
	02	408	169*
	03	409	240*
	04	390	223
	05	362	194*
	06	334	146*
	08	398	219*
	09	276	118*
	X1	410	175*
	X2	405	227*

Run Day	Win	n	TPs
TUBRUN 01	01	405	190*
	02	402	187*
	03	274	127*
	04	392	229
	05	352	176*
	06	340	157*
	08	316	157*
	09	9	1*
	X1	381	185*
	X2	378	192*

Run Day	Win	n	TPs
TUBRUN 10	01	402	198*
	02	405	199*
	03	260	124*
	04	384	224
	05	329	147*
	06	266	124*
	08	268	114*
	09	9	0*
	X1	372	186*
	X2	371	191*

Results are presented for sample days of each run; in each case the time series is that of activity level in named windows. n is the number of independent data points, with (*) indicating a significant ($P < 0.05$) departure from random expectation. For explanation see Table A.0.6, windows are identified in Table 4.2.3

Table B.0.5

Run	*Partition*	*Obs*	*Exp*
BIGRUN	*1*	*96*	*117.3*
(293)	*2*	*67*	*70.5*
	3	*51*	*42.4*
	4	*33*	*25.5*
	5	*21*	*15.3*
	6	*12*	*9.2*
	7	*13*	*12*

Run	*Partition*	*Obs*	*Exp*
MIDRUN	*1*	*114*	*128.5*
(270)	*2*	*69*	*67.5*
	3	*49*	*35.4*
	4	*22*	*18.6*
	5	*11*	*9.7*
	6	*3*	*5.1*
	7	*2*	*5.2*

Run	*Partition*	*Obs*	*Exp*
SMARUN	*1*	*101*	*120.5*
(263)	*2*	*79*	*65.4*
	3	*34*	*35.5*
	4	*28*	*19.2*
	5	*8*	*10.4*
	6	*8*	*5.7*
	7	*5*	*6.1*

Run	*Partition*	*Obs*	*Exp*
LITRUN	*1*	*91*	*119.3*
(277)	*2*	*71*	*68.0*
	3	*53*	*38.8*
	4	*32*	*22.1*
	5	*15*	*12.6*
	6	*8*	*7.2*
	7	*7*	*8.5*

Run	*Partition*	*Obs*	*Exp*
DIFRUN	*1*	*87*	*107.7*
(265)	*2*	*80*	*64.0*
	3	*34*	*38.1*
	4	*28*	*22.6*
	5	*15*	*13.5*
	6	*12*	*7.0*
	7	*9*	*10.3*

Run	*Partition*	*Obs*	*Exp*
TUBRUN	*1*	*72*	*75.5*
(218)	*2*	*32*	*49.4*
	3	*40*	*32.3*
	4	*18*	*21.1*
	5	*28*	*13.8*
	6	*13*	*9.0*
	7	*15*	*14.0*

Observed and expected frequencies of cycles for goodness of fit test. As discussed in Section A.4, goodness of fit to a geometric distribution was tested by generating an expected distribution based on the frequency of events in 10 partitions. In order to produce expected frequencies > 5*, the last (longest duration) three partitions were amalgamated (to produce partition 7). Range of duration is equal in partitions 1 to 6; absolute duration increases from partition 1 to partition 7. Obs is the number of observed cycles within a partition; Exp is the number expected (from*

the maximum likelihood distribution) from the model. Total number of cycles is in parenthesis below the run name.

Table B.0.6

Run	Day	1	2	3	4	5
BIGRUN	03	1	0	0	0	0
	04	2	1	0	0	0
	05	1	1	2	1	2
	06	3	0	0	0	0
	07	1	1	0	0	0
	08	1	0	0	0	0
	09	1	3	0	0	0
	10	3	0	0	0	0
	11	1	0	0	0	0
	12	0	0	0	0	0
	13	0	0	0	0	0
	14	0	0	0	0	0
	15	0	0	0	0	0
	16	0	0	0	0	0
	17	0	0	0	0	0
	18	0	0	0	0	0

Run	Day	1	2	3	4	5
MIDRUN	01	0	1	0	0	0
	02	0	0	0	0	0
	03	1	0	0	0	0
	04	0	0	0	0	0
	05	1	1	0	0	0
	06	0	0	0	0	0
	07	0	0	0	0	0
	08	0	0	0	0	0
	09	0	0	0	2	0
	10	4	1	0	0	0
	11	1	0	0	0	0
	12	1	0	0	0	0
	13	0	0	0	0	0
	14	3	0	1	0	0
	15	0	0	0	0	0
	16	0	1	1	0	0

Run	Day	1	2	3	4	5
SMARUN	01	1	2	0	0	0
	02	1	1	0	0	0
	03	0	1	0	0	0
	04	1	1	0	0	0
	05	1	0	0	0	0
	06	0	0	0	0	0
	07	1	0	0	0	0
	08	1	0	0	0	1
	09	1	0	0	0	0
	10	0	0	0	0	0
	11	0	0	0	0	0
	12	0	0	0	0	0
	13	1	0	0	0	0
	14	0	0	0	0	0
	15	1	0	0	0	0

Run	Day	1	2	3	4	5
LITRUN	01	1	0	2	0	0
	02	1	0	1	0	0
	03	0	1	0	0	0
	04	0	0	0	0	0
	05	0	0	0	0	0
	06	0	1	0	0	0
	07	0	0	0	0	0
	08	4	0	1	0	0
	09	0	0	0	0	0
	10	1	0	0	0	0
	11	0	0	1	0	0
	12	1	0	0	0	0
	13	0	1	0	0	0
	14	0	0	0	0	0
	15	0	1	0	0	0
	16	1	0	1	0	0

Run	Day	1	2	3	4	5
DIFRUN	01	1	0	0	0	0
	02	0	0	0	0	0
	03	0	1	1	0	0
	04	3	0	0	0	0
	05	1	0	0	0	0
	06	0	0	0	0	0
	07	1	0	1	0	0
	08	0	1	0	0	0
	09	2	0	0	0	0
	10	0	0	0	0	1
	11	1	0	0	0	0
	12	1	0	0	0	0
	13	0	0	0	0	0
	14	0	1	0	0	0
	15	1	1	0	0	0
	16	2	0	0	0	0
	17	1	1	0	0	0

Run	Day	1	2	3	4	5
TUBRUN	01	1	1	0	0	0
	02	0	1	0	0	1
	03	0	0	2	0	3
	04	0	4	1	1	1
	05	0	0	0	0	0
	06	0	0	0	0	2
	07	0	1	0	0	0
	10	0	0	1	0	1
	11	0	0	0	0	0
	12	0	0	0	0	0
	13	0	0	0	0	0
	14	0	0	1	0	0
	15	1	0	0	0	0
	16	0	0	0	0	0
	17	1	0	0	0	0
	19	0	0	0	0	0

Number of short cycles in daily time series runs. For each time series in Chapter 4, the number of cycles that are shorter than s (minimum cycle length estimated from the model) is given in columns 1 to 5, which indicate the number of cycles that fall within the range: $s - x$ to $s - (x - 1)$, where x is column number (in minutes).

Table B.0.7

*Turning point analysis of daily time series from Chapter 6. Results are presented for each day of each run. n is the number of independent data points, TPs (obs) is the number of observed turning points. * indicates a significant ($P < 0.05$) departure from random expectation. + indicates curtailed time series, 95% confidence interval for this day are 187 to 202 turning points. For explanation see Table A.0.6.*

Run	*Day*	*n*	*TPs (obs)*
STARVE	*01*	*411*	*211**
	02	*407*	*217**
	03	*410*	*222**
	04	*404*	*218**
	05	*408*	*201**
	06	*411*	*231**
	07	*413*	*213**
	08	*414*	*216**
	10	*410*	*196**
	11	*414*	*196**
	12	*414*	*201**
	13	*411*	*212**
	14	*408*	*217**
	15	*411*	*192**
	16	*419*	*195**
	17	*414*	*218**
	18	*409*	*192**
	19	*408*	*201**
	20	*411*	*201**
	21	*411*	*190**
	22	*410*	*204**
	23	*413*	*196**
	24	*413*	*204**
	25	*409*	*201**
	26	*411*	*197**
	27	*408*	*209**
	28	*406*	*197**
	29	*409*	*213**
	30	*410*	*225**
	31	*410*	*215**
	32	*294+*	*164**
	33	*413*	*229**
	34	*411*	*234**
	35	*410*	*209**
	36	*412*	*220**
	37	*411*	*211**
	38	*412*	*232**
	39	*413*	*211**
	40	*408*	*198**
	42	*404*	*205**
	43	*411*	*212**

Run	*Day*	*n*	*TPs (obs)*
2STARVE	*01*	*411*	*196**
	03	*408*	*213**
	04	*415*	*204**
	05	*411*	*204**
	06	*409*	*202**
	07	*406*	*192**
	08	*406*	*208**
	09	*408*	*199**
	10	*410*	*189**
	11	*406*	*192**
	12	*409*	*193**
	13	*405*	*170**
	14	*408*	*174**
	15	*412*	*187**
	16	*410*	*167**
	17	*408*	*164**
	18	*409*	*167**
	19	*411*	*162**
	20	*411*	*182**
	21	*411*	*165**
	22	*413*	*196**
	23	*412*	*195**
	24	*412*	*186**
	25	*411*	*185**
	26	*405*	*180**
	28	*411*	*179**
	29	*409*	*194**
	30	*413*	*218**
	34	*411*	*194**
	35	*413*	*226**
	36	*411*	*210**
	37	*411*	*197**
	38	*409*	*206**
	39	*413*	*205**
	40	*413*	*215**
	41	*411*	*206**
	42	*412*	*192**
	43	*407*	*190**
	44	*411*	*210**
	45	*412*	*209**
	46	*405*	*226**

Table B.0.8

Run	Day	n	TPs (obs)	LCI	UCI
ZIGRUN	01	412	245*	264	282
ZIG	01	296	171*	188	204
	02	299	165*	190	206
	03	292	162*	186	201
	04	298	170*	190	205
	05	296	158*	188	204
	06	295	169*	188	203

*Turning points for runs ZIGRUN and ZIG ('linear nest'), as discussed in Section 7.1. * indicates significant ($P < 0.05$) departure from random expectation. n, number of independent data points; TPs (obs) number of observed turning points. LCI, UCI indicate 95% confidence interval on random expectation. ZIGRUN: 415 frames taken at 1 minute intervals. ZIG: 300 frames taken at 10 second intervals.*

Table B.0.9

Run	Day	n	TPS (obs)
1POK	01	414	196*
	02	413	213*
	03	416	224*
	04	417	222*
	05	415	212*
	06	416	214*
	07	413	208*
	08	407	203*
	09	411	223*
	10	413	213*
	11	411	224*
	12	412	236*
	13	416	205*
	14	417	217*
	15	415	222*
	16	417	230*

Run	Day	n	TPs (obs)
GRID	01	413	208*
	02	409	206 *
	03	411	227*
	04	415	239*
	05	415	242*
	06	412	236*
	07	414	240*
	08	414	212*

Run	Day	n	TPs (obs)
BOX	01		
	02	412	219*
	03	414	231*
	04		
	05	415	234*
	06	411	203*
	07	411	221*
	08	416	221*
	09	415	241*
	10	412	221*
	11	409	204*
	12	412	217*

*Turning points for runs 1POK, GRID and BOX. n is the number of independent observations; TPs (obs) is the number of observed turning points. Those marked * depart significantly ($P < 0.05$) from random; for details see Table A.0.6*

Table B.0.10

Chamber	Day	n	TPs (obs)
1	01	393	178*
	02	401	189*
	03	401	169*
	04	392	191*
	05	391	189*
	06	382	165*
	07	390	193*
	08	387	202*
	09	388	194*
	10	389	197*
	11	384	193*
	12	396	194*
	13	382	178*
	14	394	201*
	15	379	171*
	16	376	166*

Chamber	Day	n	TPs (obs)
2	01	402	216*
	02	394	191*
	03	388	193*
	04	391	186*
	05	387	173*
	06	390	178*
	07	395	190*
	08	379	163*
	09	398	195*
	10	400	211*
	11	392	194*
	12	397	200*
	13	390	191*
	14	392	181*
	15	391	201*
	16	388	191*

Chamber	Day	n	TPs (obs)
3	01	395	191*
	02	396	186*
	03	394	207*
	04	387	172*
	05	383	174*
	06	395	194*
	07	396	183*
	08	390	188*
	09	403	208*
	10	399	199*
	11	386	181*
	12	401	198*
	13	387	178*
	14	399	191*
	15	395	190*
	16	395	174*

Chamber	Day	n	TPs (obs)
4	01	398	184*
	02	379	160*
	03	398	199*
	04	395	189*
	05	391	186*
	06	388	182*
	07	391	183*
	08	399	206*
	09	397	205*
	10	391	182*
	11	396	186*
	12	392	191*
	13	397	187*
	14	401	221*
	15	397	204*
	16	394	201*

Chamber	Day	n	TPs (obs)
5	01	399	211*
	02	406	218*
	03	396	211*
	04	411	226*
	05	399	205*
	06	399	193*
	07	396	200*
	08	399	200*
	09	405	213*
	10	407	213*
	11	400	221*
	12	401	221*
	13	390	206*
	14	377	175*
	15	396	201*
	16	399	193*

Chamber	Day	n	TPs (obs)
6	01	395	189*
	02	399	209*
	03	401	201*
	04	402	194*
	05	392	184*
	06	393	170*
	07	397	189*
	08	397	187*
	09	401	186*
	10	395	192*
	11	399	195*
	12	398	194*
	13	399	193*
	14	400	198*
	15	397	186*
	16	398	195*

Chamber	Day	n	TPs (obs)
7	01	402	205*
	02	392	191*
	03	384	155*
	04	394	199*
	05	403	194*
	06	395	160*
	07	393	163*
	08	394	194*
	09	401	192*
	10	389	191*
	11	406	202*
	12	386	180*
	13	388	188*
	14	396	214*
	15	390	202*
	16	401	209*

Chamber	Day	n	TPs (obs)
8	01	397	212*
	02	401	210*
	03	396	196*
	04	402	200*
	05	403	198*
	06	392	183*
	07	394	207*
	08	401	197*
	09	398	203*
	10	399	174*
	11	391	188*
	12	398	212*
	13	398	212*
	14	394	206*
	15	398	179*
	16	399	208*

*Turning points for chambers within the multichambered nest n, number of independent data points, TPs (obs) number of observed turning points. * indicates significant departure ($P< 0.05$) from random expectation, for details see Table A.0.6.*

Table B.0.11

1POK

Win	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
1	*-4*	*0*	*1*	*3*	*-1*	*1*	*0*	*-2*	*5**	*0*	*2*	*2*
2		*0*	*2*	*8**	*6**	*2*	*-1*	*0*	*8**	*9**	*3*	*2*
3			*1*	*2*	*6**	*2*	*3*	*1*	*3*	*4*	*6**	*2*
4				*-1*	*-2*	*0*	*-1*	*0*	*-1*	*2*	*1*	*5**
5					*0*	*-2*	*2*	*1*	*11**	*-1*	*-1*	*3*
6						*3*	*3*	*0*	*3*	*7**	*3*	*2*
7							*2*	*4*	*1*	*2*	*7**	*7**
8								*0*	*3*	*-2*	*1*	*15**
9									*6**	*5**	*2*	*3*
10										*13**	*2*	*2*
11											*9**	*-1*
12												*14**

GRID

Win	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
1	*5**	*1*	*0*	*7**	*0*	*2*	*0*	*2*	*-1*	*-1*	*1*	*4**
2		*6**	*4**	*0*	*1*	*1*	*1*	*2*	*1*	*0*	*1*	*0*
3			*4**	*1*	*1*	*0*	*2*	*0*	*0*	*-1*	*-1*	*-1*
4				*0*	*0*	*-1*	*8**	*-1*	*1*	*0*	*2*	*3*
5					*3*	*1*	*0*	*7**	*0*	*-1*	*0*	*8**
6						*0*	*-1*	*4**	*4**	*0*	*-3*	*0*
7							*1*	*0*	*-3*	*0*	*-1*	*1*
8								*0*	*-1*	*2*	*6**	*0*
9									*3*	*-2*	*2*	*3*
10										*5**	*0*	*-1*
11											*5**	*0*
12												*1*

BOX

Win	2	3	4	5	6	7	8	9	10	11	12	13
1	8*	1	2	10*	-1	2	0	3	0	1	-1	1
2		9*	3	2	5*	1	0	1	0	0	2	0
3			5*	2	1	3	2	-1	-2	-1	-1	0
4				1	0	2	10*	0	1	0	8*	2
5					3	0	-2	3	-2	1	-1	2
6						0	-3	0	2	-1	-3	1
7							5*	1	2	5*	2	0
8								2	-1	3	8*	2
9									10*	5*	-1	6*
10										10*	1	4*
11											7*	4*
12												6*

TUBRUN

Win	2	3	4	5	6	7	8	9	10	11	12	13
1	3	1	1	6	1	3	3	2	3	6	5	2
2		4	8*	4	6	5	8*	6	3	6	9*	3
3			14*	2	6	16*	16*	5	11*	12*	13*	4
4				4	5	15*	16*	5	9*	16*	16*	4
5					5	6	4	9*	5	9*	4	12*
6						13*	8*	5	12*	13*	6	1
7							16*	7*	11*	16*	16*	1
8								12*	12*	16*	16*	4
9									13*	13*	10*	6
10										16*	12*	3
11											16*	6
12												3

*Correlation scores for regions within nests, results from Chapter 7. Tables present the number of significant correlations (- indicates negative correlations) between activity time series in pairs of windows (as labelled; axes of Tables: see also Figure 7.6) over all days of the run. (16 for 1POK and TUBRUN, 8 for GRID; 10 for BOX.) Those marked * indicate a significant* ($P < 0.05$) *departure from random expectation (see Appendix A.6).*

Appendix C

Programs

C.1 ASTATA.BAS

```
Input "Number of files to read ",Maxf
Dim A$(Maxf),B(Maxf,50),Cnt(Maxf),Mn(Maxf),Vr(Maxf),Chi(Maxf)
Dim C(Maxf,50),Ctr%(11),Ev(11),Df(Maxf),Slp(Maxf),Ch2(15),Bst(5),Prb(5)
Dim F(Maxf,9),G(Maxf,9),H(Maxf,9),K(Maxf,9),L(Maxf,9),Obs1(11),Exp1(11)
Dim Zobs(11),Zexp(11)
For File=1 To Maxf
  Fileselect "\*.*",B$,A$(File)
  Open "I",#1,A$(File)
  C=0
  While Not (Eof(#1))
    Input #1,B(File,C)
    Mn(File)=Mn(File)+B(File,C)
    C=C+1
  Wend
  Close #1
  Cnt(File)=C
  Print "Mean of file ";A$(File);" = ";Mn(File)/Cnt(File)
  Mn(File)=Mn(File)/Cnt(File)
  For Cnt=0 To Cnt(File)-1
    Vr(File)=Vr(File)+(B(File,Cnt)-Mn(File))^2
  Next Cnt
  Vr(File)=Vr(File)/Cnt(File)
  Print "and Variance = ";Vr(File)
Next File
For J0=1 To Maxf
  Gosub Dochi(J0)
  Print "Chi^2 for file ";A$(J0);" is = ";Chi(J0)
```

```
Next J0
For J=1 To Maxf
  Print "Include file ",A$(J);" in final data";
  Input B$
  B$=Left$(B$,1)
  If B$="n" Or B$="N"
    Df(J)=-1
  Endif
Next J
Gosub Dostats
Gosub Calcdata
Gosub Alldata
Procedure Dochi(File)
  P1=(2+1/Vr(File)-Sqr((2+1/Vr(File))^2-4))/2
  Bst1=Mn(File)-1/(1-P1)
  Slp(File)=Bst1
  Max1=0
  For J=0 To Cnt(File)-1
    C(File,J)=B(File,J)-Bst1
    If C(File,J)>Max1
      Max1=C(File,J)
    Endif
    If C(File,J)<0
      Print "warning too small in ";File
    Endif
  Next J
  For J=1 To 11
    Ctr%(J)=0
  Next J
  For Ct=0 To Cnt(File)-1
    K=0
    Repeat
      K=K+1
    Until Max1*K/10>=C(File,Ct)
    Ctr%(K)=Ctr%(K)+1
  Next Ct
  Q1=1-P1
  For K=0 To 9
    Ev(K+1)=0
    For J=K*Max1/10 To (K+1)*Max1/10
      Ev(K+1)=Ev(K+1)+(P1^J)*Q1*Cnt(File)
    Next J
  Next K
  Chi(File)=0
  For J=1 To 10
    Chi(File)=Chi(File)+(Ctr%(J)-Ev(J))^2/Ev(J)
```

```
  Next J
Return
Procedure Dostats
  Cnt=0
  For F=1 To Maxf
    Ct=0
    If Df(F)<>-1
      While B(F,Ct)>0
        Mnw=Mnw+B(F,Ct)
        Ct=Ct+1
        Cnt=Cnt+1
      Wend
    Endif
  Next F
  Mnw=Mnw/Cnt
  For F=1 To Maxf
    Ct=0
    If Df(F)<>-1
      While B(F,Ct)>0
        Wssq=Wssq+(B(F,Ct)-Mnw)^2
        Thrd=Thrd+(B(F,Ct)-Mnw)^3
        Four=Four+(B(F,Ct)-Mnw)^4
        Ct=Ct+1
      Wend
    Endif
  Next F
  Wssq=Wssq/Cnt
  Sd=Wssq^0.5
  Thrd=Thrd/(Cnt*(Sd^3))
  Four=(Four/(Cnt*(Sd^4)))-3
  Thrd=Thrd/Cnt
  Wssql=Wssq-2*Wssq*Sqr(2/Cnt)
  Wssqm=Wssq+2*Wssq*Sqr(2/Cnt)
  Prb(1)=(2+1/Wssq-Sqr((2+1/Wssq)^2-4))/2
  Prb(2)=(2+1/Wssqm-Sqr((2+1/Wssqm)^2-4))/2
  Prb(3)=(2+1/Wssql-Sqr((2+1/Wssql)^2-4))/2
  Bst(1)=Mnw-1/(1-Prb(1))
  Bst(2)=Mnw-1/(1-Prb(3))+2*Sqr(Wssqm/Cnt)
  Bst(3)=Mnw-1/(1-Prb(2))-2*Sqr(Wssql/Cnt)
  Dim A%(11),Pr(11)
  X=1
  For F1=1 To 3
    For F2=1 To 3
      Ch2=0
      Gosub Normalise(Bst(F1),Prb(F2))
      Print "Possible chi^2 for all=";Ch2
```

```
      Ch2((F1-1)*3+F2)=Ch2
      If F1=1
        If F2=1
          For J=1 To 10
            Obs1(J)=Zobs(J)
            Exp1(J)=Zexp(J)
          Next J
        Endif
      Endif
      X=X+1
    Next F2
  Next F1
Return
Procedure Normalise(Sleep,P)
  Maxv=0
  For F=0 To 11
    A%(F)=0
    Pr(F)=0
  Next F
  For F=1 To Maxf
    Ct=0
    If Df(F)<>-1
      While B(F,Ct)>0
        C(F,Ct)=B(F,Ct)-Sleep
        If C(F,Ct)=<-60 And C(F,Ct)>-120
          F(F,X)=F(F,X)+1
        Endif
        If C(F,Ct)=<-120 And C(F,Ct)>-180
          G(F,X)=G(F,X)+1
        Endif
        If C(F,Ct)=<-180 And C(F,Ct)>-240
          H(F,X)=H(F,X)+1
        Endif
        If C(F,Ct)=<-240 And C(F,Ct)>-300
          K(F,X)=K(F,X)+1
        Endif
        If C(F,Ct)=<-300
          L(F,X)=L(F,X)+1
        Endif
        If C(F,Ct)>Maxv
          Maxv=C(F,Ct)
        Endif
        Ct=Ct+1
      Wend
    Endif
  Next F
```

```
  For F=1 To Maxf
    Ct=0
    If Df(F)<>-1
      For Ct=0 To Cnt(F)-1
        K=0
        Repeat
          K=K+1
        Until Maxv*K/10>=C(F,Ct)
        A%(K)=A%(K)+1
      Next Ct
    Endif
  Next F
  Q=1-P
  For K=0 To 9
    For J=K*Maxv/10 To (K+1)*Maxv/10
      Pr(K+1)=Pr(K+1)+(P^J)*Q*Cnt
    Next J
  Next K
  Gosub Chicalc
Return
Procedure Chicalc
  For J=1 To 7
    Zexp(J)=Pr(J)
    Zobs(J)=A%(J)
    If J=7
      Zexp(J)=Zexp(J)+Pr(J+1)+Pr(J+2)+Pr(J+3)
      Zobs(J)=Zobs(J)+A%(J+1)+A%(J+2)+A%(J+3)
    Endif
    Ch2=Ch2+(Zobs(J)-Zexp(J))^2/Zexp(J)
  Next J
Return
Procedure Calcdata
  Big=0
  Lit=0
  Clen=Int(Mnw*100+0.5)/100
  Cerr=Int(Sqr(Wssq/Cnt)*196+0.5)/100
  Slen=Clen-Cerr
  Blen=Clen+Cerr
  Sma=0
  Big=0
  For File=1 To Maxf
    If Df(File)<>-1
      For C=1 To Cnt(File)
        If B(File,C)<Slen
          Lit=Lit+1
        Endif
```

```
        If B(File,C)>Blen
          Big=Big+1
        Endif
      Next C
    Endif
  Next File
Return
Procedure Alldata
  Start:
  Cls
  For J=1 To Maxf
    Print "Mean = ";Int(Mn(J)+0.5);" Variance = ";Int(Vr(J)+0.5);" Chi^2 = ";
Int(Chi(J)+0.5);
    Print " sleep time =";Int(Slp(J)+0.5);" #cycles = ";Cnt(J)
  Next J
  Print
  Print "Mean of whole = ";Int(Mnw*100+0.5)/100;" variance = ";
Int(Wssq*100+0.5)/100;
  Print " Chi^2 = ";Int(Ch2(1)*100+0.5)/100;"  sleep time = ";
Int(Bst(1)*100+0.5)/100
  Print "Third moment = ";Thrd^(1/3);"   Total # of cycles = ";Cnt
  Print "mean cycle length in mins =";Int(Mnw*100+0.5)/6000
  Print "sleep time in mins=";Int(Bst(1)*100+0.5)/6000
  Print "Error range = ";Int(Sqr(Wssq/Cnt)*196+0.5)/100
  Print "Possible values for chi^2 =";
  For F=2 To 9
    If F=5
      Print
      Print "          ";
    Endif
    Print Int(Ch2(F)*100+0.5)/100;"    ";
  Next F
  Print
  Print
  Print "no. of cycles below range is ";Lit
  Print
  Print "no. of cycles above range is ";Big
  Print
  Print "g1 (3rd moment) = ";Thrd
  Print "g2 (4th moment) = ";Four
  Print
  Print "observed and expected totals for partitions (main chi):"
  For J=1 To 10
    Print "cell ";J;"   obs ";Obs1(J);"       expected ";Exp1(J)
  Next J
  Print
```

```
  Input "HARDCOPY?",X$
  If X$="y" Or X$="Y"
    Gosub Hall
  Endif
  Input "info on cycle mismatches ?",Z$
  If Z$="Y" Or Z$="y" Then
    Gosub Leninf
  Else
    Goto Start
  Endif
Return
Procedure Leninf
  Cls
  Print "problem(X) = no. cycles where (length-sleep) < -Xmins"
  Print "                                        and >-(X-1)mins"
  Print
  Print "probs for main chi^2 are: "
  J=1
  Reveal:
  Print "              prob1  prob2  prob3  prob4  prob5"
  Print
  For File=1 To Maxf
    If File<10
      Print "file  ";File;"        ";F(File,J);"       ";G(File,J);"      ";
H(File,J);"      ";K(File,J);"      ";L(File,J)
    Endif
    If File>=10
      Print "file ";File;"        ";F(File,J);"       ";G(File,J);"      ";
H(File,J);"      ";K(File,J);"      ";L(File,J)
    Endif
  Next File
  Input "hardcopy?",H$
  If H$="y" Or H$="Y"
    Gosub Hlen
  Endif
  Input "more info? ",M$
  If M$="Y" Or M$="y"
    Input "enter chi^2 no. for further probs ",J
    Goto Reveal
  Endif
  Input "Q to quit",Q$
  If Q$="Q" Or Q$="q" Then
    End
  Else
    Gosub Alldata
  Endif
```

```
Return
Procedure Hall
  For J=1 To Maxf
    Lprint "Mean = ";Int(Mn(J)+0.5);" Variance = ";Int(Vr(J)+0.5);" Chi^2 = "
Int(Chi(J)+0.5);
    Lprint " sleep time =";Int(Slp(J)+0.5);" #cycles = ";Cnt(J)
  Next J
  Lprint
  Lprint "Mean of whole = ";Int(Mnw*100+0.5)/100;" variance = ";
Int(Wssq*100+0.5)/100;
  Lprint " Chi^2 = ";Int(Ch2(1)*100+0.5)/100;"  sleep time = ";
Int(Bst(1)*100+0.5)/100
  Lprint "Total # of cycles = ";Cnt;"   third moment = ";Thrd^(1/3)
  Lprint "mean cycle length in mins =";Int(Mnw*100+0.5)/6000
  Lprint "sleep time in mins=";Int(Bst(1)*100+0.5)/6000
  Lprint "Error range = ";Int(Sqr(Wssq/Cnt)*196+0.5)/100
  Lprint "Possible values for chi^2 =";
  For F=2 To 9
    If F=5
      Lprint
      Lprint "          ";
    Endif
    Lprint Int(Ch2(F)*100+0.5)/100;"    ";
  Next F
  Lprint
  Lprint
  Lprint "no. of cycles below range is ";Lit
  Lprint
  Lprint "no. of cycles above range is ";Big
  Lprint
  Lprint "g1 (3rd moment = ";Thrd
  Lprint "g2 (4th moment = ";Four
  Lprint
  Lprint "observed and expected totals for partitions (main chi):"
  For J=1 To 10
    Lprint "cell ";J;"      observed ";Obs1(J);"      expected ";Exp1(J)
  Next J
  Lprint
  Input "another hardcopy?",U$
  If U$="y" Or U$="Y"
    Gosub Alldata
  Endif
Return
Procedure Hlen
  Lprint "problem(X) = no. cycles where (length-sleep) < -Xmins"
  Lprint "                                and > -(X-1)mins"
```

```
  Lprint
  Lprint "              prob1  prob2  prob3  prob4  prob5"
  Lprint
  For File=1 To Maxf
    If File<10
      Lprint "file  ";File;"        ";F(File,J);"       ";G(File,J);"      ";
H(File,J);"      ";K(File,J);"      ";L(File,J)
    Endif
    If File>=10
      Lprint "file ";File;"        ";F(File,J);"       ";G(File,J);"      ";
H(File,J);"      ";K(File,J);"      ";L(File,J)
    Endif
  Next File
  Input "another hardcopy?",V$
  If V$="Y" Or V$="y"
    Gosub Leninf
  Endif
Return
```

C.2 SPREDLOC.BAS

```
Dim A%(600,25),B%(600),Mn%(100)
Deffn Fn1=A%(I,1)-A%(I,2)
Deffn Fn2=A%(I,6)+A%(I,5)
Deffn Fn3=A%(I,0)-A%(I,3)-A%(I,4)
Deffn Fn4=A%(I,8)+A%(I,9)
Deffn Fn5=A%(I,0)-A%(I,3)-A%(I,4)-A%(I,1)
Deffn Fn6=A%(I,1)-A%(I,2)+A%(I,8)+A%(I,9)
Maxy=390
Input "file to read from ",F$
Open "R",#1,F$
Input #1,Win
Input #1,N
Max=0
For I=0 To N-2
  For J=0 To Win
    Input #1,A%(I,J)
    If A%(I,J)>Max
      Max=A%(I,J)
    Endif
  Next J
  A%(I,16)=Fn Fn1
  A%(I,17)=Fn Fn2
  A%(I,18)=Fn Fn3
  A%(I,19)=Fn Fn4
  A%(I,20)=Fn Fn5
  A%(I,21)=Fn Fn6
Next I
While 1=1
  Point=1
  While Point>=0
    Print
    Input "Point number to edit (negative to go on)",Point
    If Point>=0
      Print "Current value is ",A%(Point);
      Input " new value ",A%(Point)
    Endif
  Wend
  Gosub Dodraw
  Print
  Input "Hard copy (q to stop)",A$
  If A$="y" Or A$="Y" Or A$="yes"
    Gosub Dodraw
    Hardcopy
```

```
  Endif
  If A$="q"
    End
  Endif
  W1=0
  While W1>=0
    Input "Window for analysis ",W1
    If W1>=0 Then
      Width=10
      While Width>=0
        Print
        Input "Width for minima ",Width
        If Width>=0
          For I=Width To N-Width-1
            Min=10000
            For J=I-Width To I+Width
              If A%(J,W1)<Min
                Min=A%(J,W1)
              Endif
            Next J
            B%(I)=Min
          Next I
          Gosub Do1draw
          Print
          Gosub Getminima
          Input "Hard copy (q to stop)",A$
          If A$="y" Or A$="Y" Or A$="yes"
            Gosub Do1draw
            Hardcopy
          Endif
          If A$="q"
            End
          Endif
        Endif
      Wend
    Endif
  Wend
Wend
Procedure Dodraw
  Input "Window value to draw",W
  Max=0
  For I=0 To N
    If A%(I,W)>Max
      Max=A%(I,W)
    Endif
  Next I
```

```
  Cls
  If Max=0 Then
    Max=1
  Endif
  Color 1
  Draw 10,Maxy-10 To 460,Maxy-10
  Draw 10,10 To 10,Maxy-10
  Draw 7,10 To 13,10
  Print At(3,1);Max;
  Draw 7,Maxy/2+10 To 13,Maxy/2+10
  Print At(3,13);Int(Max/2)
  For I=1 To 6
    Draw I*75+10,Maxy-12 To I*75+10,Maxy-8
    Print At(I*9.5+1,24);Int(N/6*I);
  Next I
  For I=0 To N-2
    Draw I*450/N+10,(Maxy-10-(A%(I,W)*(Maxy-10)/Max)) To
(I+1)*450/N+10,(Maxy-10-(A%(I+1,W)*(Maxy-10)/Max))
  Next I
Return
Procedure Do1draw
  Max=0
  For I=Width To N-Width-2
    If B%(I)>Max
      Max=B%(I)
    Endif
  Next I
  Cls
  If Max=0 Then
    Max=1
  Endif
  Color 1
  Draw 10,Maxy-10 To 460,Maxy-10
  Draw 10,10 To 10,Maxy-10
  Draw 7,10 To 13,10
  Print At(3,1);Max;
  Draw 7,Maxy/2+10 To 13,Maxy/2+10
  Print At(3,13);Int(Max/2)
  For I=1 To 6
    Draw I*75+10,Maxy-12 To I*75+10,Maxy-8
    Print At(I*9.5+1,24);Int(N/6*I);
  Next I
  For I=Width To N-Width-2
    Draw I*450/N+10,(Maxy-(B%(I)*(Maxy-10)/Max)-10) To
(I+1)*450/N+10,(Maxy-10-(B%(I+1)*(Maxy-10)/Max))
  Next I
```

```
Return
Procedure Getminima
  Mins=0
  Px=0
  Mouse Mx,My,K
  While K<>2
    Mouse Mx,My,K
    If K=1
      Cx=Mx
      Flag=1
    Endif
    If K=0 And Flag=1
      Mn%(Mins)=Mx
      Flag=0
      Mins=Mins+1
      Draw Mx,My-3 To Mx,My+3
    Endif
  Wend
  Input "Save to file ",C$
  If C$="y"
    Input "File name",F1$
    Open "O",#2,F1$
    Input "Seconds interval per frame ",S
    Spp=N*S/460
    For X=1 To Mins-1
      Len=(Mn%(X)-Mn%(X-1))*Spp
      If Len<=0
        Print "warning: dodgy value ignored"
        Goto Abort
      Endif
      Print #2,Len
      Abort:
    Next X
    Close #2
  Endif
Return
```

C.3 NC1.BAS

```
Rem iterval between frames
Time%=60
Rem total time in minutes
Long%=420
Rem Base file name
File1$="D:\ant00001"
Rem buffer for screen
Dim Buf%(16000)
Dummy%=Bios(12345,L:Varptr(I%),0,0,0)
If (Dummy% And &HFFFF)=&HFFFF
  Print "Realtizer not connected to a video signal"
  Stop
Endif
If ((Dummy% And &HFFFF)=12345)
  Print "DQUIUTI.PRG is not loaded"
  Stop
Endif
Kont%=I% And &HFFFF
Hell%=I% Div 65536
Rem these are fixed values set be me may need to change!!
Hell%=119
Kont%=114
Print "Finished Autosetting",Hell%,Kont%
Dim Z%(1000),Dummy%(5,2,2,2,2)
Rem ******** digitize ********
Gray%=34
Dummy%=Bios(12345,L:Xbios(3),Gray%,Hell%,Kont%)
Rem -----------saving a screen to disc-----------
X%=1
Sto%=Int((Long%/Time%)*60)
For Inc%=1 To Sto%
  T=Timer
  Basename$=Left$(File1$,Len(File1$)-X%)
  Dummy%=Bios(12345,L:Xbios(3),Gray%,Hell%,Kont%)
  Rem Bmove Varptr(Buf%(0)),Xbios(3),32000
  Screen$=Basename$+Str$(Inc%)
  Bsave Screen$,Xbios(3),32000
  If Inc%>8 Then
    X%=2
  Endif
  If Inc%>98 Then
    X%=3
  Endif
```

```
  Repeat
  Until (Timer-T)>=Time%*200
Next Inc%
End
```

C.4 amw1.c

```
#include <e:\clang\headers\stdio.h>
#include <e:\clang\headers\fcntl.h>
#include <e:\clang\headers\portab.h>
#include <e:\clang\headers\gemlib.h>
#include <e:\clang\headers\stdlib.h>
#include <linea.h>

short scale,thr,thr1,thr2,prt;
char b1[32000],b2[32000],a1[32000],a2[32000];
int fhan,numw,maxx[100],maxy[100],minx[100],miny[100];


main()
{ char c,b[10];
  scale=4;thr=8;thr1=8;thr2=12;prt=0; /* default values */
  numw=0;minx[0]=0;miny[0]=0;maxx[0]=640;maxy[0]=500;
  linea0();
  printf("Good morning Campers!!\n\n");
  help();
  while (b[0]!='e')
   { printf("\n\nOption:");
     getstring(b);
     if (b[0]=='r') rd_b1();
     if (b[0]=='R') rd_b2();
     if (b[0]=='d') displayfile(a1);
     if (b[0]=='D') displayfile(a2);
     if (b[0]=='b') blk_b1();
     if (b[0]=='B') blk_b2();
     if (b[0]=='h') help();
     if (b[0]=='c') compare();
     if (b[0]=='s') getparams();
     if (b[0]=='a') analyse();
     if (b[0]=='p') st_print();
     if (b[0]=='w') disp_win();
     if (b[0]=='S') write_params();
     if (b[0]=='l') load_params();
   }
}

disp_win()
{ int j;
  displayfile(a1);
  for (j=0;j<=numw;j++)
```

```
  {
  drw_hline(minx[j],miny[j],maxx[j],miny[j],1);
  drw_vline(maxx[j],miny[j],maxx[j],maxy[j],1);
  drw_hline(maxx[j],maxy[j],minx[j],maxy[j],1);
  drw_vline(minx[j],maxy[j],minx[j],miny[j],1);
  };
}

st_print()
{ char b[100];
  printf("file to send data to ?");
  getstring(b);
  fhan=creat(b,0);
  printf("\nOutput will be sent to printer and file until the
end of analysis.");
  prt=100;prtnum(numw);
}

help()
{printf("e to exit\nr to read buffer 1\nR to read buffer 2\n");
 printf("d to diplay buffer 1\nD to display buffer 2\n");
 printf("c to compare buffer 1 and 2\nb to block buffer 1\n");
 printf("B to block buffer 2\ns to reset parameters\n");
 printf("p to save output of comparisons\n");
 printf("a to analyse a block of files\nh to get this message back\n");
 printf("w to display current window");
 printf("\nS to save parameters\nl to load parameters");

}


getstring(b)
char *b;
{ int i;
  char c;
  for (i=0;(i<100) && ((c=getchar()) !=EOF) && (c!='\n'); ++i)
  b[i]=c;
  b[i]= '\0';
}

getparams()
{ char b[100];
  int j;
  short x;
  printf("Block size is %d set to ? ",scale);
  getstring(b);
```

```
  x=atoi(b);
  if (x !=0 ) scale=x;
  printf("Block drawing threshold is %d set to ? ",thr);
  getstring(b);
  x=atoi(b);
  if (x!=0) thr=x;
  printf("Lower comparison threshold is %d set to ? ",thr1);
  getstring(b);
  x=atoi(b);
  if (x!=0) thr1=x;
  printf("Upper comparison threshold is %d set to ? ",thr2);
  getstring(b);
  x=atoi(b);
  if (x!=0) thr2=x;
  printf("Number of windows is %d set to ? ", numw);
  getstring(b);
  x=atoi(b);

  if (x!=0) numw=x;
  if (numw>0)
  { for (j=1;j<=numw;j++)
    {
      printf("Minimum value for x is %d in window %d set to ?",minx[j],j);
      getstring(b);x=atoi(b);
      if (x!=0) minx[j]=x;
      printf("Minimum value for y is %d in window %d set to ?",miny[j],j);
      getstring(b);x=atoi(b);
      if (x!=0) miny[j]=x;
      printf("Maximum value for x is %d in window %d set to ?",maxx[j],j);
      getstring(b);x=atoi(b);
      if (x!=0) maxx[j]=x;
      printf("Maximum value for y is %d in window %d set to ?",maxy[j],j);
      getstring(b);x=atoi(b);
      if (x!=0) maxy[j]=x;
     };
   };
}

write_params()
{ char b[100];
  int j;

  printf("File to save parameters in ?");
  getstring(b);
  fhan=creat(b,0);
  prtnum(scale);
```

```
  prtnum(thr);
  prtnum(thr1);
  prtnum(thr2);
  prtnum(numw);
  if (numw>0)
  { for (j=1;j<=numw;j++)
    {
      prtnum(minx[j]);
      prtnum(miny[j]);
      prtnum(maxx[j]);
      prtnum(maxy[j]);
     };
   };
   close(fhan);
}

int gtnum()
{ char b[100],c[1];
  int i;
  for (i=0;(i<100) && (read(fhan,c,1) !=0) && (c[0] !='\n');i++) b[i]=c[0];
  b[i]='\0';
  i=atoi(b);
  return i;
}


load_params()
{ char b[100];
  int j;

  printf("File to load parameters from ?");
  getstring(b);
  fhan=open(b,O_RDONLY,0);
  scale=gtnum();
  thr=gtnum();
  thr1=gtnum();
  thr2=gtnum();
  numw=gtnum();
  if (numw>0)
  { for (j=1;j<=numw;j++)
    {
      minx[j]=gtnum();
      miny[j]=gtnum();
      maxx[j]=gtnum();
      maxy[j]=gtnum();
     };
```

```
   };
   close(fhan);
}

blk_b1()
{ block_file(a1,b1);
  draw_block(b1);
}

blk_b2()
{ block_file(a2,b2);
  draw_block(b2);
}



rd_b1()
{ char b[100];
  printf("File name for buffer 1 ");
  getstring(b) ;
  getfile(a1,b);
}

rd_b2()
{ char b[100];
  printf("File name for buffer 2 ");
  getstring(b) ;
  getfile(a2,b);
}

analyse()
{ int frms,i;
  char b[100];

  frms=0;
  while ( (frms==0) || (frms>800))
  { printf("Number of frames to read ? ");
    getstring(b);
    frms=atoi(b);
  }
  if (prt>0)
    { prtnum(frms);};
  printf("Filename to start from ? ");
  getstring(b);
  getfile(a1,b);
  block_file(a1,b1);
```

```
  incstring(b);
  getfile(a2,b);
  block_file(a2,b2);
  incstring(b);
  frms=frms-2;
  compare();
  for (i=frms;i>0;i=i-2)
   { getfile(a1,b);
     block_file(a1,b1);
     compare();
     incstring(b);
     getfile(a2,b);
     block_file(a2,b2);
     compare();
     incstring(b);
   }
  printf("It's all over folks\n");
  if (prt!=0) close(fhan);
  prt=0;

}

incstring(b)
char *b;
{ int x;
  x=0;
  while (b[x]!='\0') x++;
  x=x-1;

  b[x]=b[x]+1;
  while (b[x]==('9'+1))
   { b[x]='0';x=x-1;b[x]=b[x]+1;};
}

getfile(a,b)
char *a,*b;
{ int f,j;
  printf("reading file %s\n",b);
  f=open(b,O_RDONLY,0);
  j=read(f,a,32000);
  printf("bytes read %d\n",j);
  close(f);
}

displayfile(a)
char *a;
```

```
{ short x,y,i,j;
  x=0;y=0;
  for (i=0;i<32000;i++)
   { for (j=7;j>=0;j=j-1)
      {
        if (a[i]&(1<<j))
          drw_pnt(x,y,0);
        else drw_pnt(x,y,1);
      x++;
      if (x==640) {x=0;y=y+1;};
      };
   };
}

block_file(a,b)
char *a,*b;
{ short x,y,i,j;
  int z;

  for (z=0;z<32001;z++) b[z]=0;
  x=0;y=0;
  for (i=0;i<32000;i++)
   { for (j=7;j>=0;j=j-1)
      {
        if (a[i]&(1<<j))
         {z=(x/scale)+((y/scale)*(640/scale+1));
          b[z]=b[z]+1;};
      x++;
      if (x==640) {x=0;y=y+1;};
      };
   };
}

draw_block(b)
char *b;
{ short x,y;
  for (x=0;x<(640/scale);x++)
   for (y=0;y<(500/scale);y++)
    if (b[x+(y*(640/scale+1))]>thr)
       drw_bl(x,y,1);
    else drw_bl(x,y,0);
}

compare()
{ short x,y;
  int k,j,wrongs[11];
```

```
  for (j=0;j<=numw;j++)
  {
   wrongs[j]=0;
   for (x=minx[j]/scale;x<(maxx[j]/scale);x++)
   for (y=miny[j]/scale;y<(maxy[j]/scale);y++)
    {k=x+(y*(640/scale+1));
     if ((b1[k]>thr2) &&  (b2[k]<thr1))
        { wrongs[j]=wrongs[j]+1;sdrw_bl(x,y);};
     if ((b1[k]<thr1) && (b2[k]>thr2))
        { wrongs[j]=wrongs[j]+1;sdrw_bl(x,y);};
    };
  };

  printf("Number of failures =");
  for (j=0;j<=numw;j++) printf("%d ",wrongs[j]);
  printf("\n");
  if (prt!=0)
   { fputs( "Movements = ",stdprt);
     for (j=0;j<=numw;j++) {lprint(wrongs[j]);prtnum(wrongs[j]);};
     fputs("\n",stdprt);fflush(stdprt);
   };
}

lprint(n)
int n;
{ char b[7];
  b[6]='\0';
  b[5]=' ';b[4]=(n%10)+'0';n=n/10;
  b[3]=(n % 10) + '0';n=n/10;b[2]=(n%10) + '0';
  n=n/10;b[1]=(n%10) + '0';n=n/10;b[0]=(n%10)+'0';
  fputs(b,stdprt);

}


drw_bl(x,y,c)
short x,y,c;
{ short i,j,k;

  j=y*scale;k=j+scale;
  for (i=x*scale;i<(x*scale+scale);i++)
   drw_line(i,j,i,k,c);
}
```

```
sdrw_bl(x,y)
short x,y;
{ short i,j,k;
  j=y*scale;k=j+scale;
  for (i=x*scale;i<(x*scale+scale);i=i+2)
   {drw_line(i,j,i,k,0);drw_line(i+1,j,i+1,k,1);};
}


printfile(a)
char *a;
{ int j;
  for (j=1; j<32000; j=j+100) printf ("%d;",a[j]);
}

prtnum(n)
int n;
{ char b[6];
  b[4]=n%10+'0';n=n/10;b[3]=n%10+'0';n=n/10;b[2]=n%10+'0';n=n/10;
  b[1]=n%10+'0';n=n/10;b[0]=n%10+'0';b[5]='\n';
  write(fhan,b,6);
}

drw_hline(x,y,x1,y1)
int x,y,x1,y1;
{ int j,k;
  if (x>x1) {k=x;x=x1,x1=k;};
  for (j=x;j<=x1;j=j+2)
    { drw_pnt(j,y,0);drw_pnt(j+1,y,1);};
}

drw_vline(x,y,x1,y1)
int x,y,x1,y1;
{ int j,k;
  if (y>y1) {k=y;y=y1;y1=k;};
  for (j=y;j<=y1;j=j+2)
    { drw_pnt(x,j,0);drw_pnt(x,j+1,1);};
}


drw_pnt(inx,iny,nc)
short inx,iny,nc;
{ X1=inx;Y1=iny;X2=inx;Y2=iny;
  COLBIT0=nc;COLBIT1=nc;COLBIT2=nc;COLBIT3=nc;
  WMODE=0;
  linea3();
```

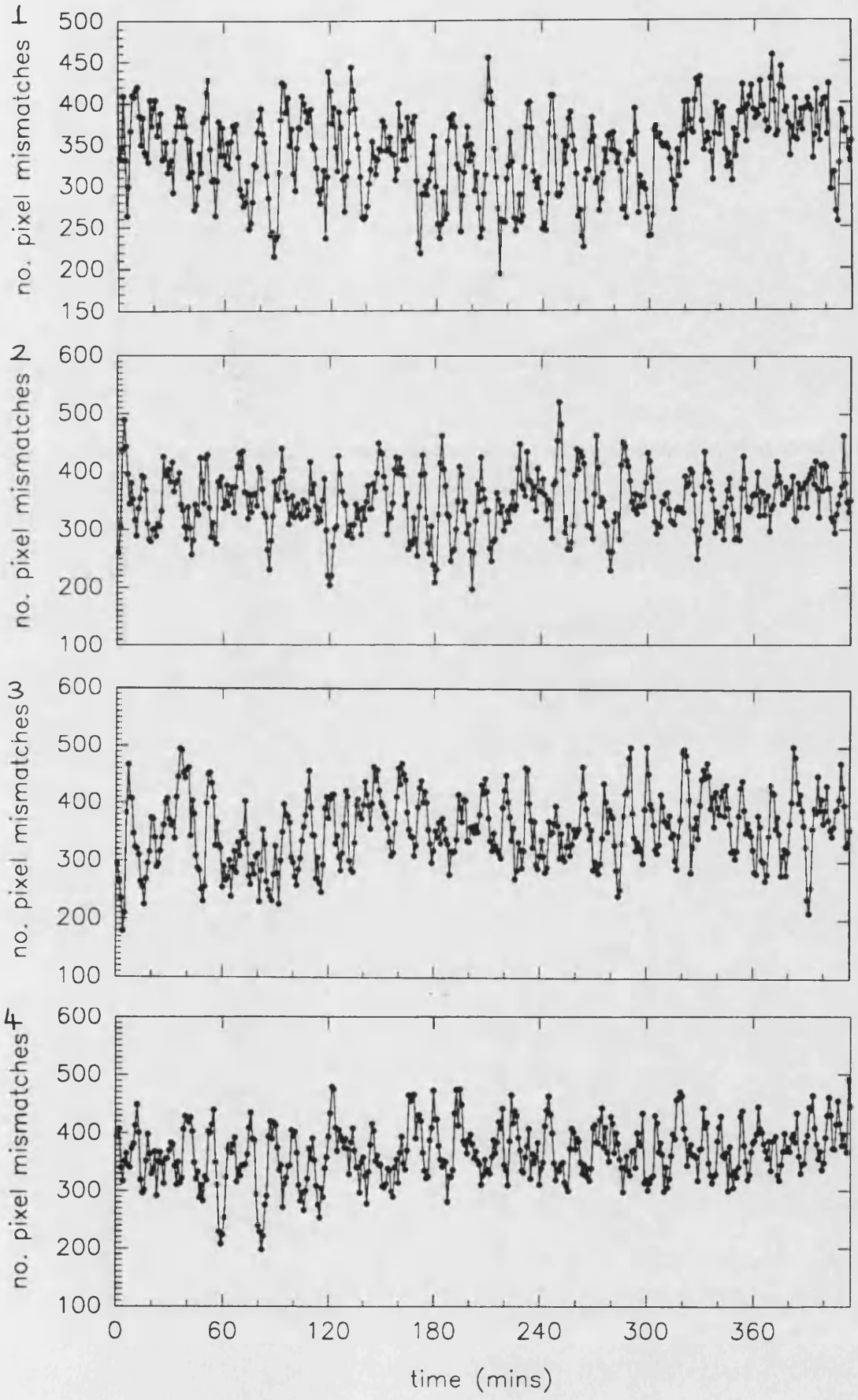
```
}

drw_line(inx,iny,inx1,iny1,nc)
short inx,iny,inx1,iny1,nc;
{ X1=inx;Y1=iny;X2=inx1;Y2=iny1;
  COLBIT0=nc;COLBIT1=nc;COLBIT2=nc;COLBIT3=nc;
  WMODE=0;
  linea3();
}
```

Appendix D

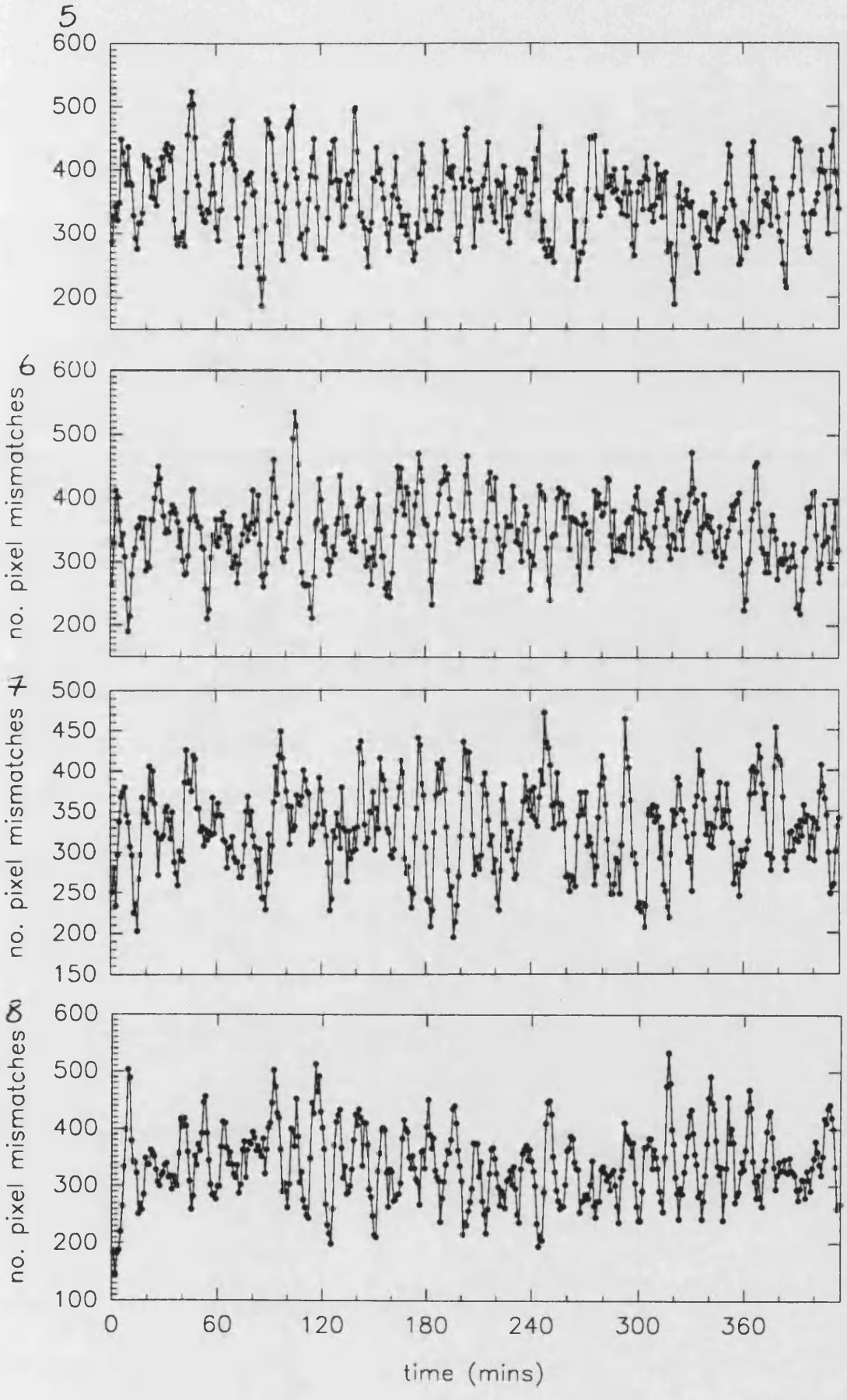
Figures

D.1 Time Series: Whole Field of View

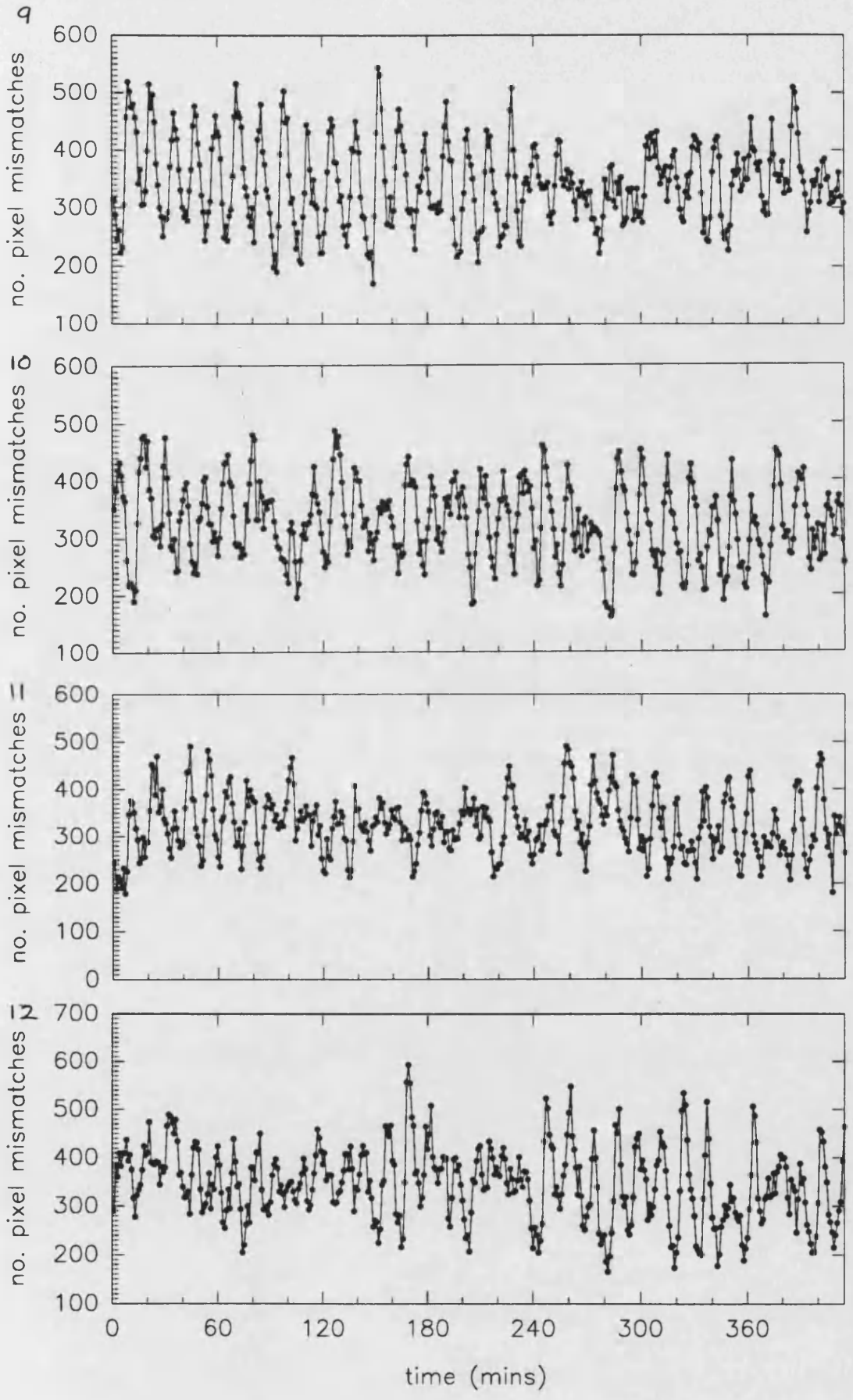
Time series of activity in the whole field of view: BIGRUN. Activity is measured as the number of pixel mismatches between consecutive frames taken at one minute intervals in window X0. Graphs 1-16 indicate daily time series for the whole run (BIGRUN03-BIGRUN18 respectively).


1
no. pixel mismatches
500
450
400
350
300
250
200
150
2
no. pixel mismatches
600
500
400
300
200
100
3
no. pixel mismatches
600
500
400
300
200
100
4
no. pixel mismatches
600
500
400
300
200
100
0
60
120
180
240
300
360
time (mins)

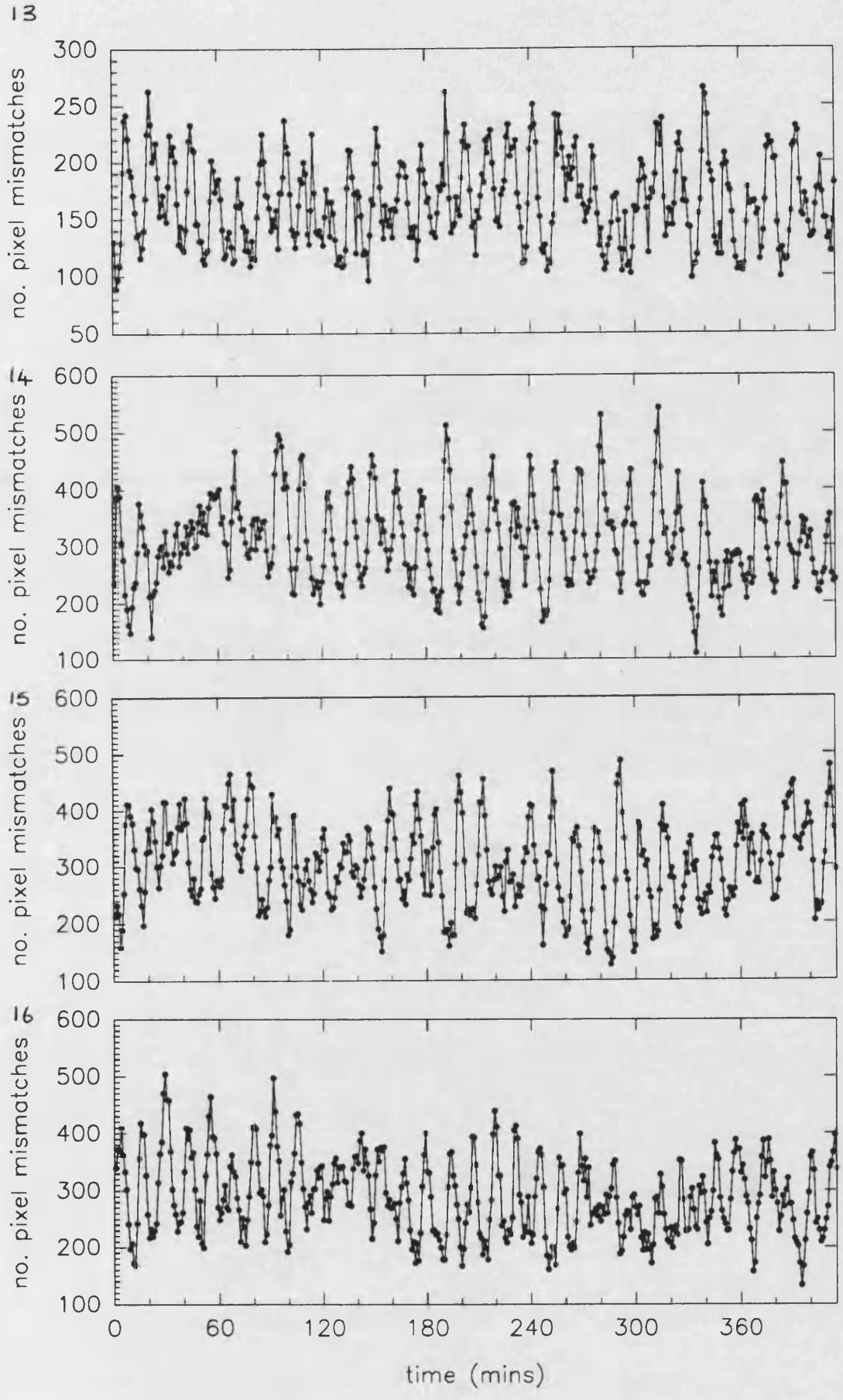
361 a


5
600
500
400
300
200
6
no. pixel mismatches
7
450
350
250
150
8
100
0
60
120
180
240
300
360
time (mins)

361b

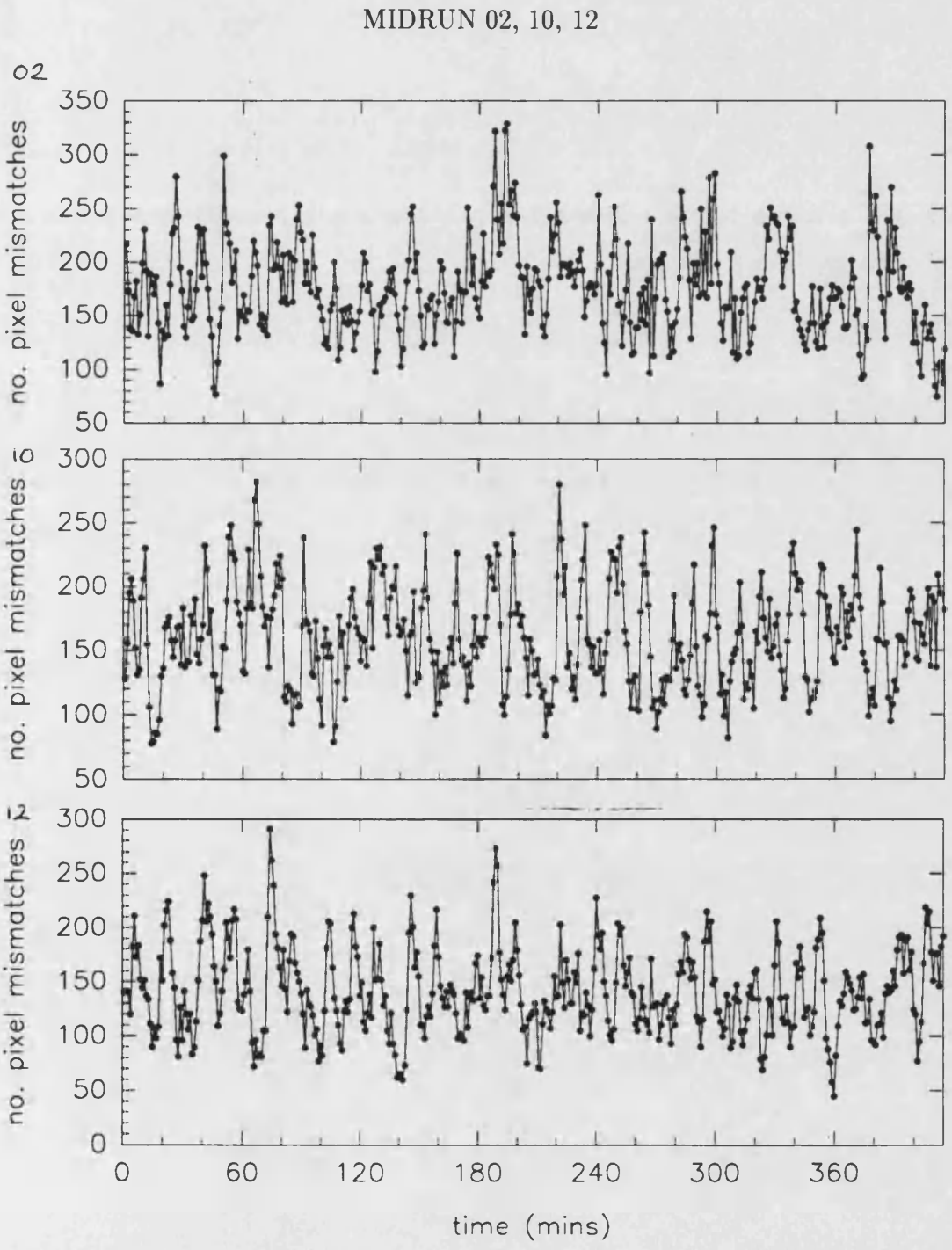

9
no. pixel mismatches
600
500
400
300
200
100
10
no. pixel mismatches
600
500
400
300
200
100
11
no. pixel mismatches
600
500
400
300
200
100
0
12
no. pixel mismatches
700
600
500
400
300
200
100
0
60
120
180
240
300
360
time (mins)

361 c

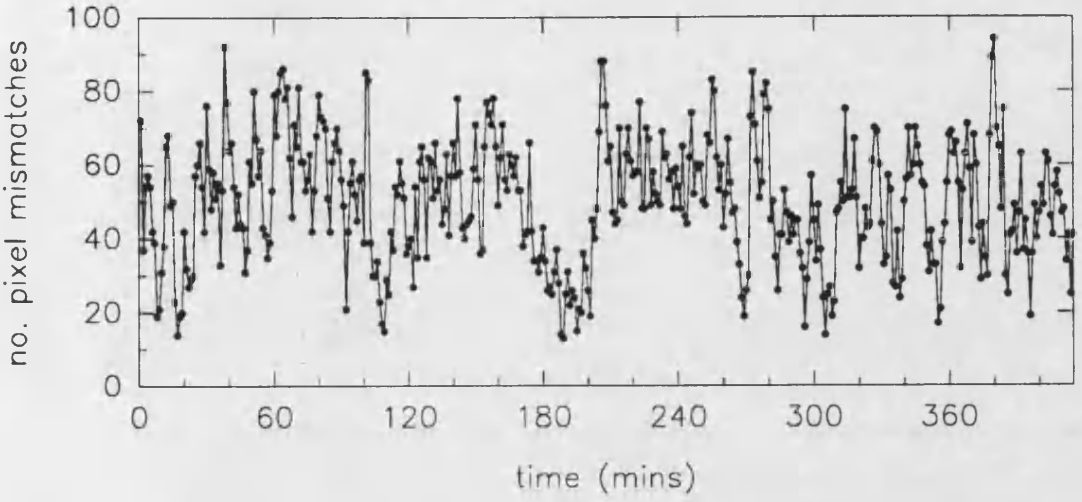

13
14
15
16
no. pixel mismatches
300
250
200
150
100
50
600
500
400
300
200
100
0
60
120
180
240
300
360
time (mins)

361 d

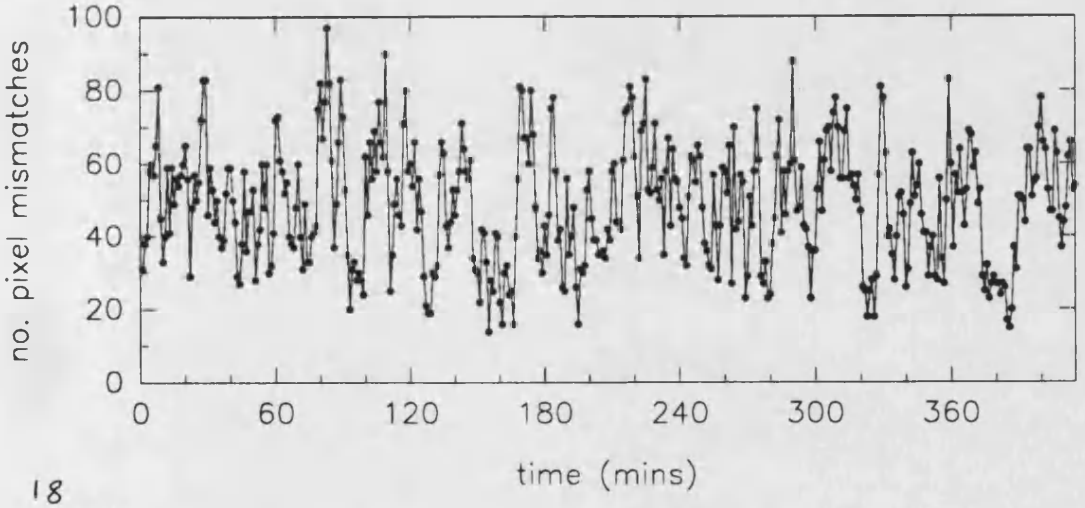
Sample time series of activity in the whole field of view: Experiments in Chapter 4. Activity is measured as the number of pixel mismatches between consecutive frames taken at one minute intervals in window X0. Graphs present activity in runs and dates as labelled.



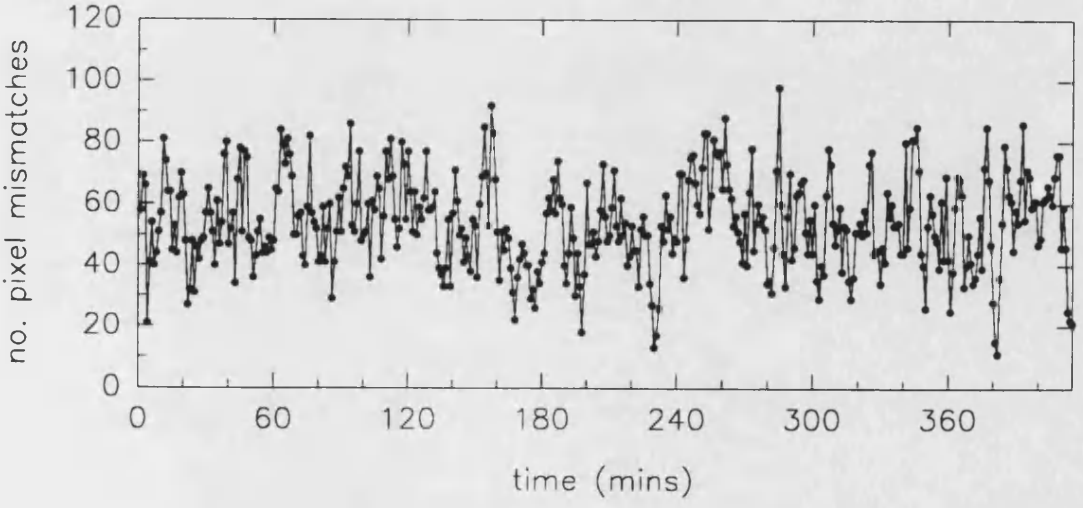
09

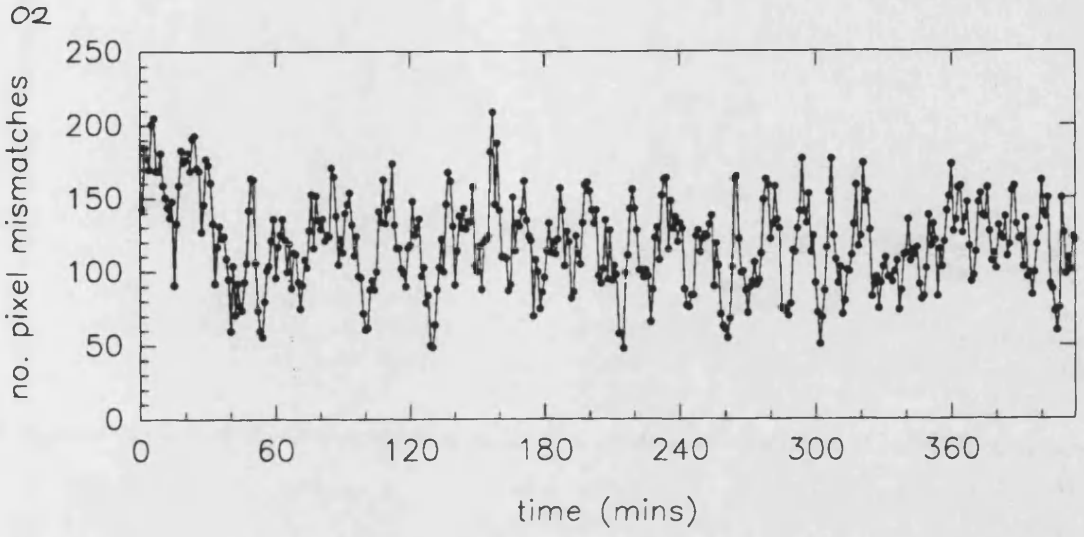

no. pixel mismatches
100
80
60
40
20
0
0
60
120
180
240
300
360
time (mins)

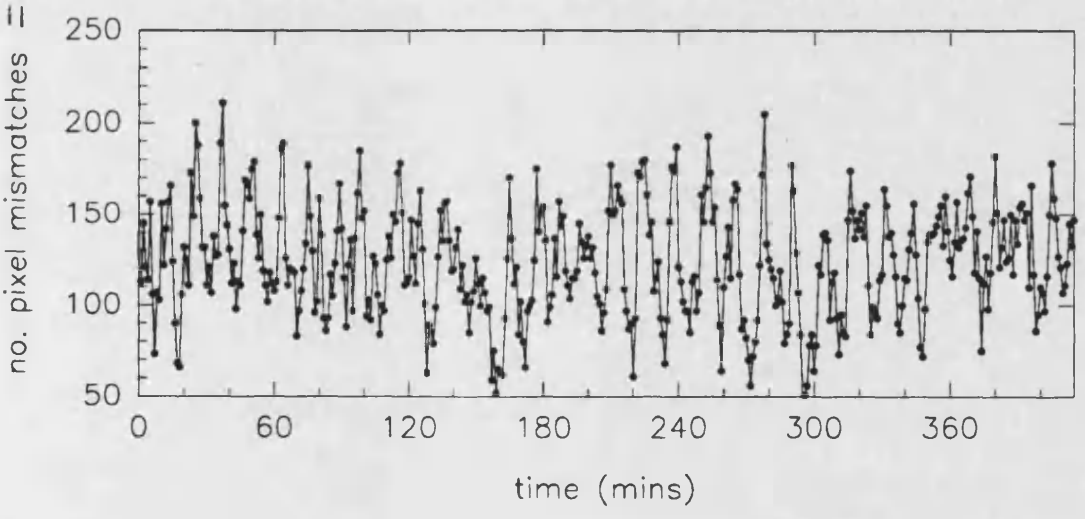
10

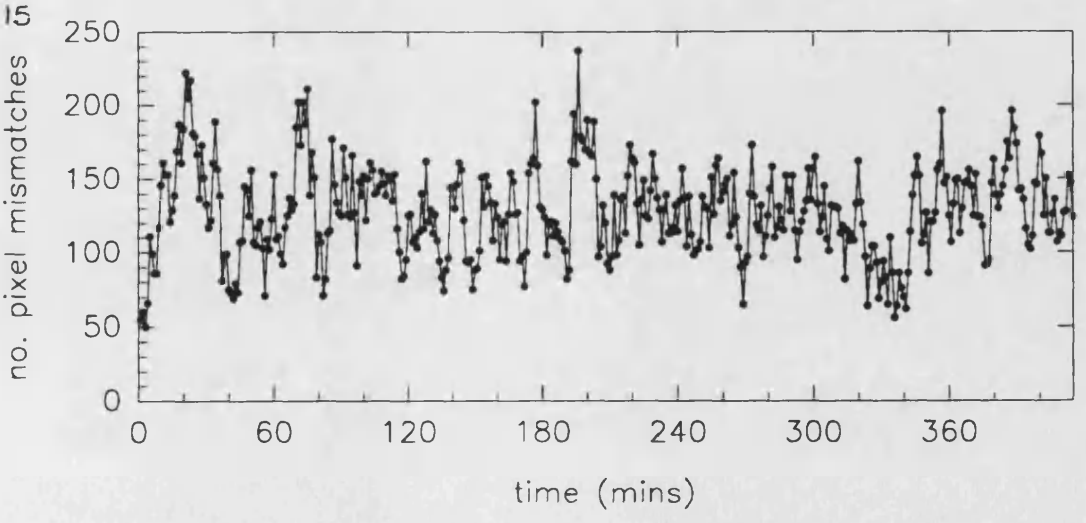

no. pixel mismatches
100
80
60
40
20
0
0
60
120
180
240
300
360
time (mins)

18

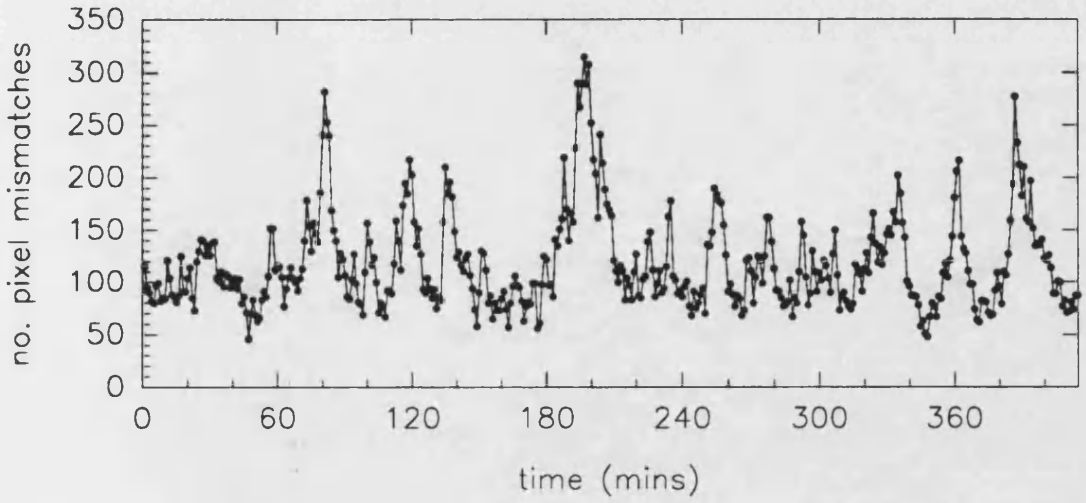

no. pixel mismatches
120
100
80
60
40
20
0
0
60
120
180
240
300
360
time (mins)


02
no. pixel mismatches
250
200
150
100
50
0
0
60
120
180
240
300
360
time (mins)

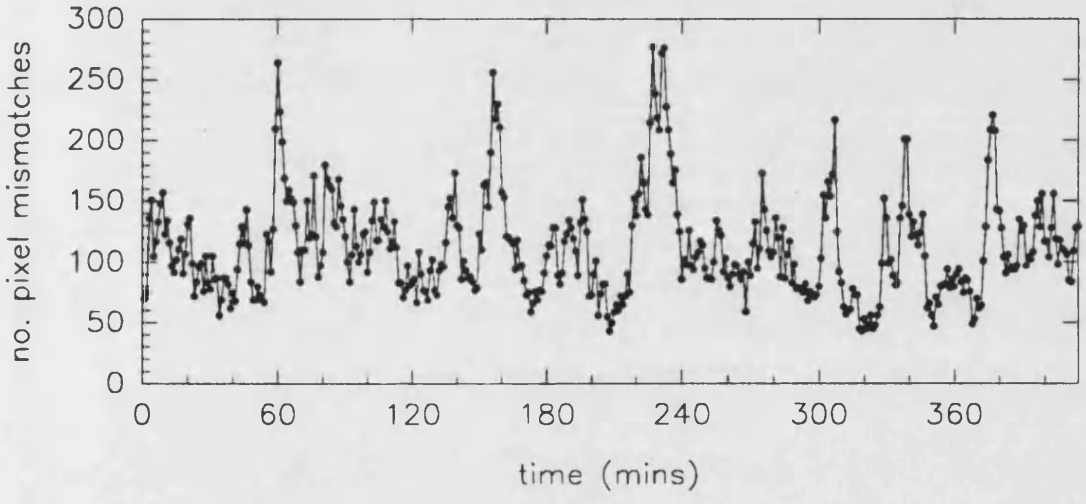

11
no. pixel mismatches
250
200
150
100
50
0
60
120
180
240
300
360
time (mins)


15
no. pixel mismatches
250
200
150
100
50
0
0
60
120
180
240
300
360
time (mins)

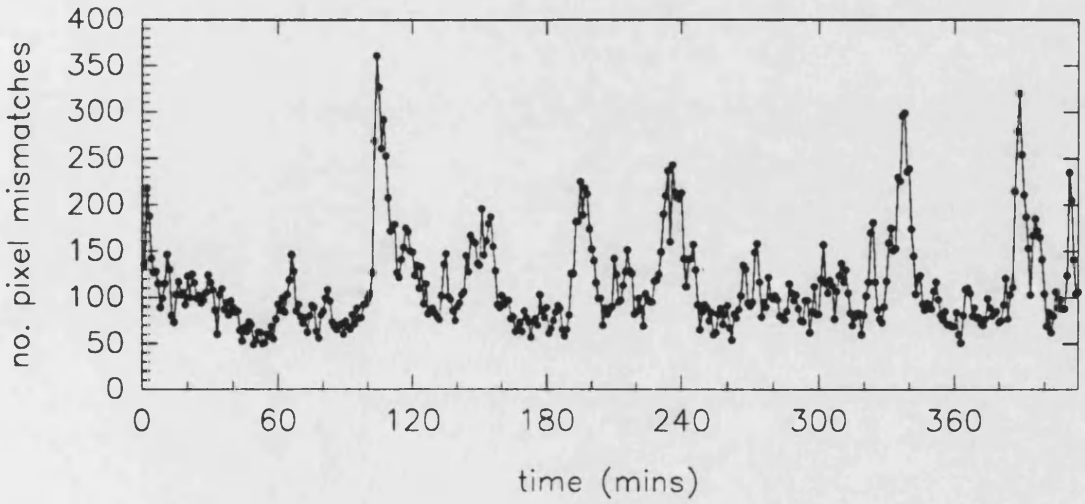
01

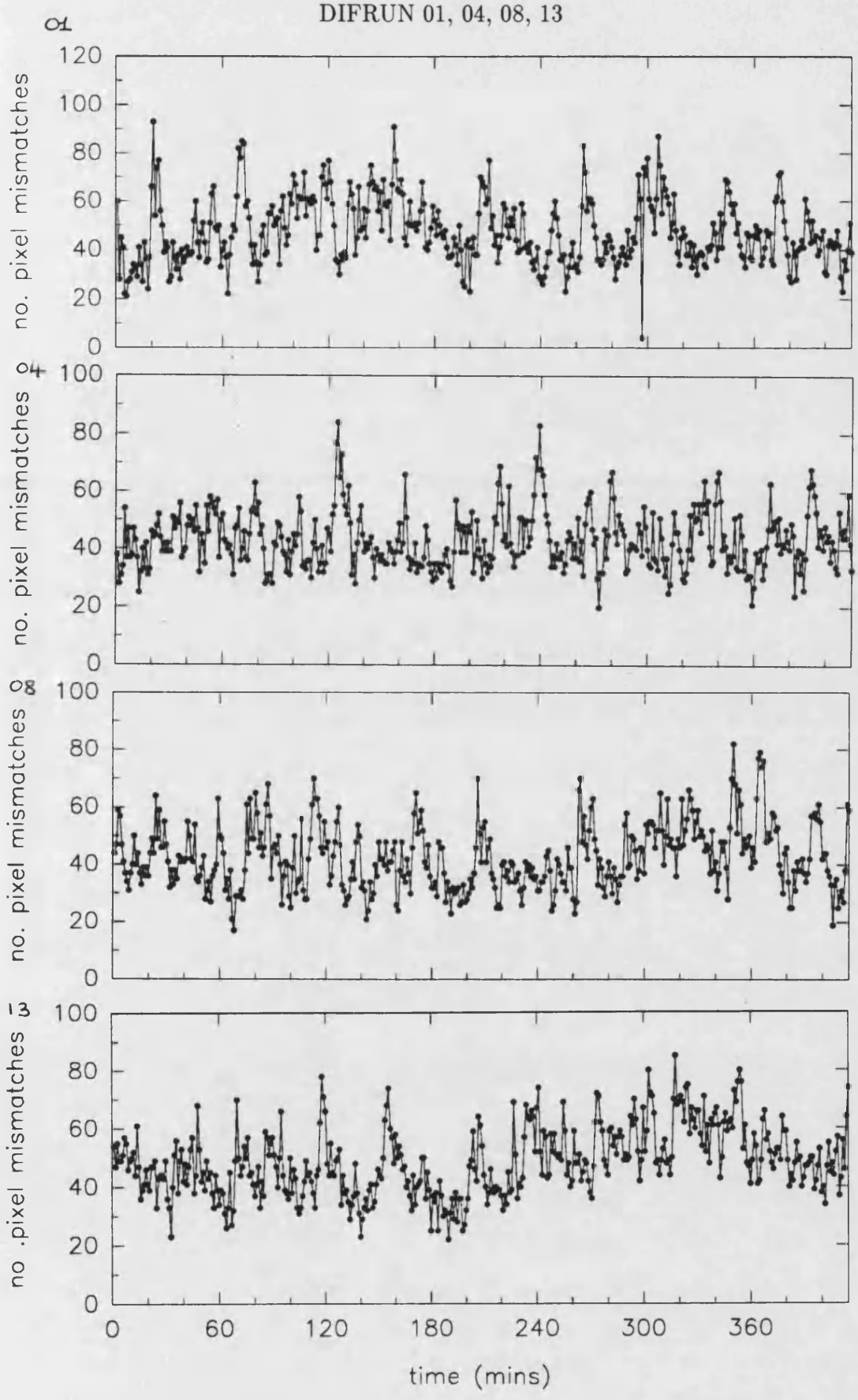

no. pixel mismatches
350
300
250
200
150
100
50
0
0
60
120
180
240
300
360
time (mins)

11

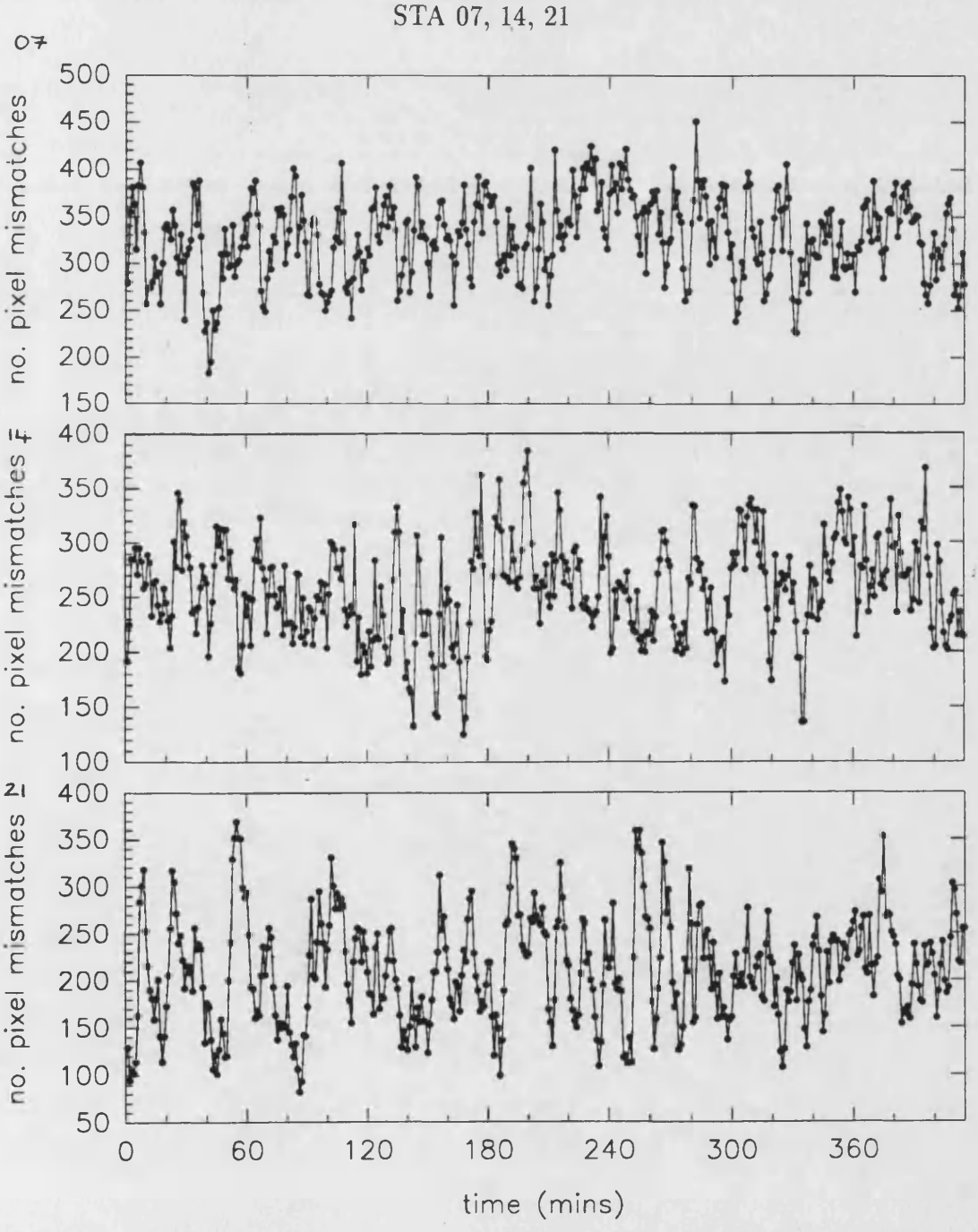

no. pixel mismatches
300
250
200
150
100
50
0
0
60
120
180
240
300
360
time (mins)

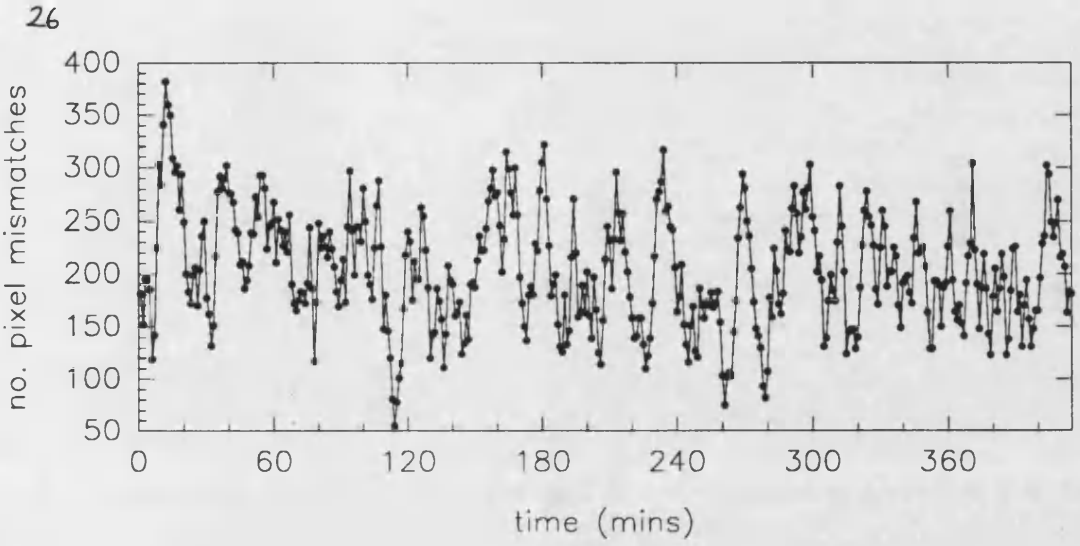
19

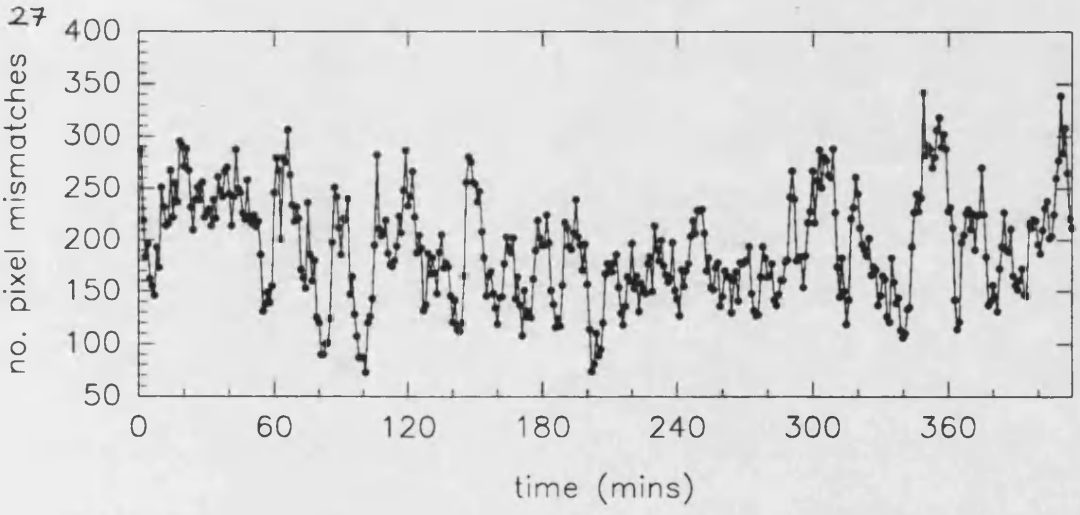

no. pixel mismatches
400
350
300
250
200
150
100
50
0
0
60
120
180
240
300
360
time (mins)

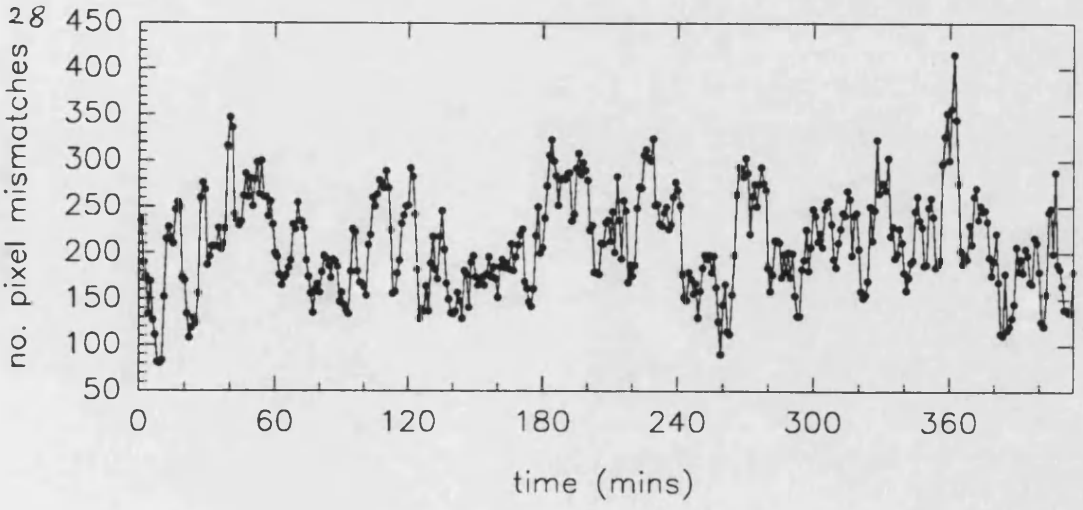

01
no. pixel mismatches
120
100
80
60
40
20
0
04
no. pixel mismatches
100
80
60
40
20
0
08
no. pixel mismatches
100
80
60
40
20
0
13
no .pixel mismatches
100
80
60
40
20
0
0
60
120
180
240
300
360
time (mins)

Sample time series of activity in the whole field of view: Experiments in Chapter 6. Activity is measured as the number of pixel mismatches between consecutive frames taken at one minute intervals in window X0. Graphs present activity in runs and dates as labelled (STA: food removed day 08, returned day 30; 2STA: food removed day 08, returned day 36).

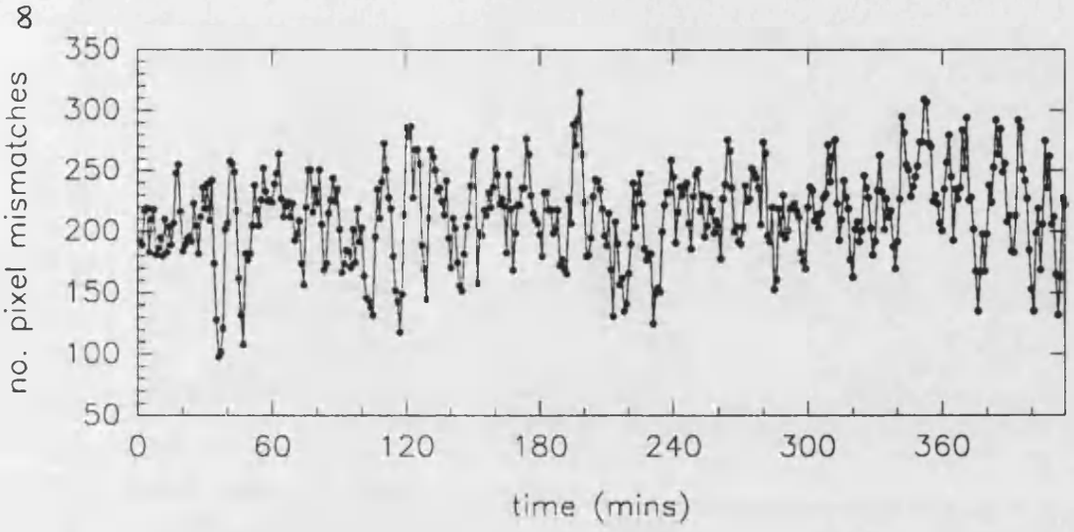



26
no. pixel mismatches
400
350
300
250
200
150
100
50
0
60
120
180
240
300
360
time (mins)

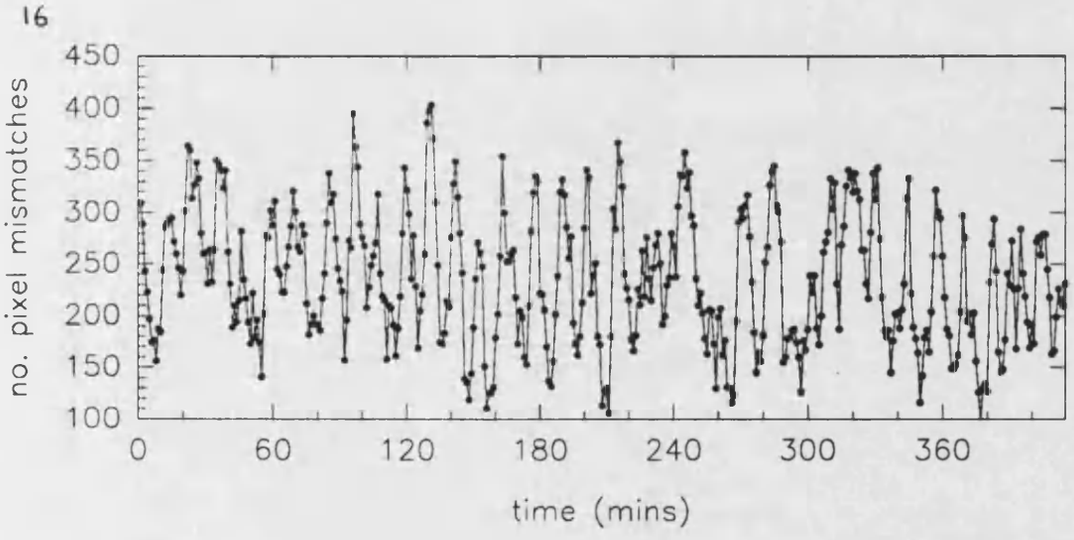

27
no. pixel mismatches
400
350
300
250
200
150
100
50
0
60
120
180
240
300
360
time (mins)


28
no. pixel mismatches
450
400
350
300
250
200
150
100
50
0
60
120
180
240
300
360
time (mins)

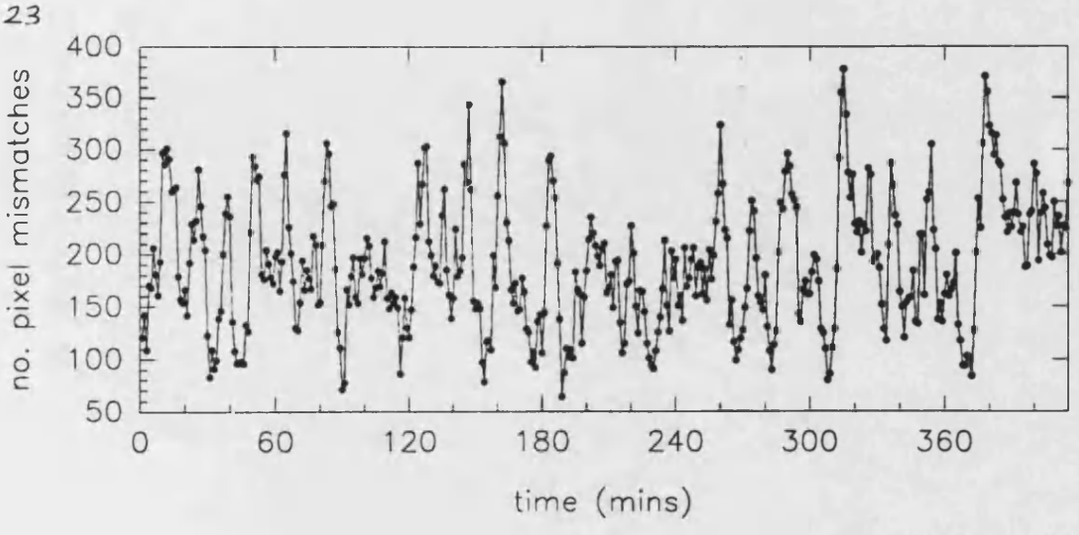
8

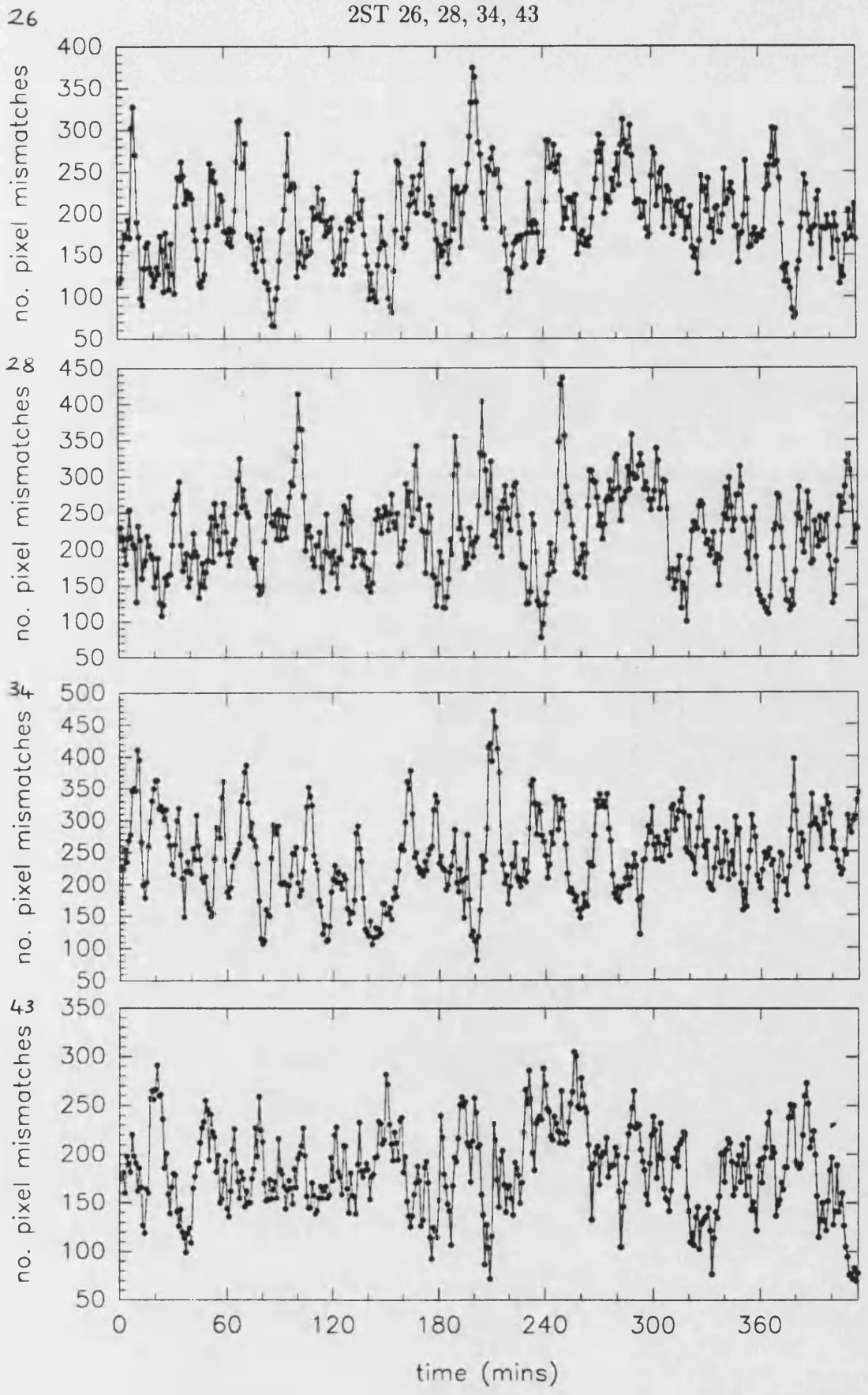

no. pixel mismatches
350
300
250
200
150
100
50
0
60
120
180
240
300
360
time (mins)

16


no. pixel mismatches
450
400
350
300
250
200
150
100
0
60
120
180
240
300
360
time (mins)

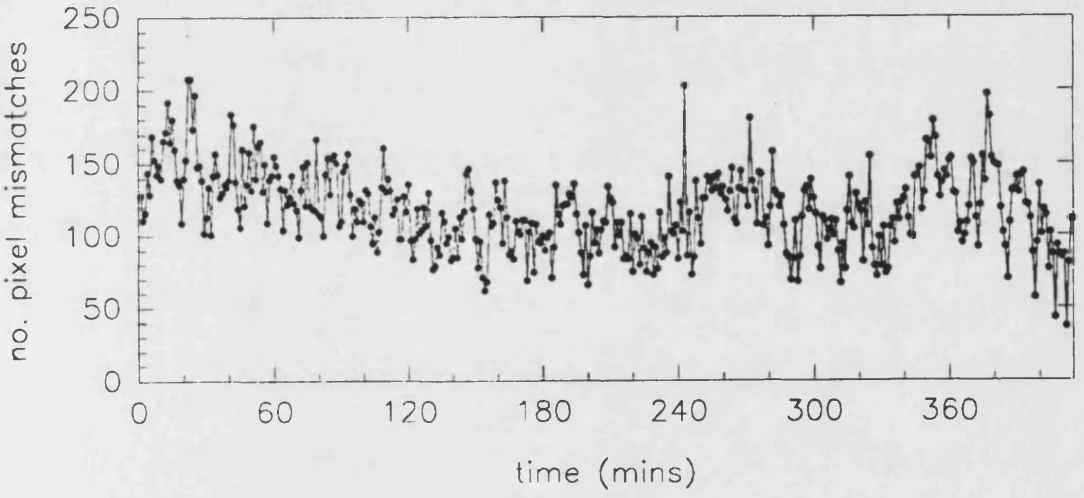
23


no. pixel mismatches
400
350
300
250
200
150
100
50
0
60
120
180
240
300
360
time (mins)

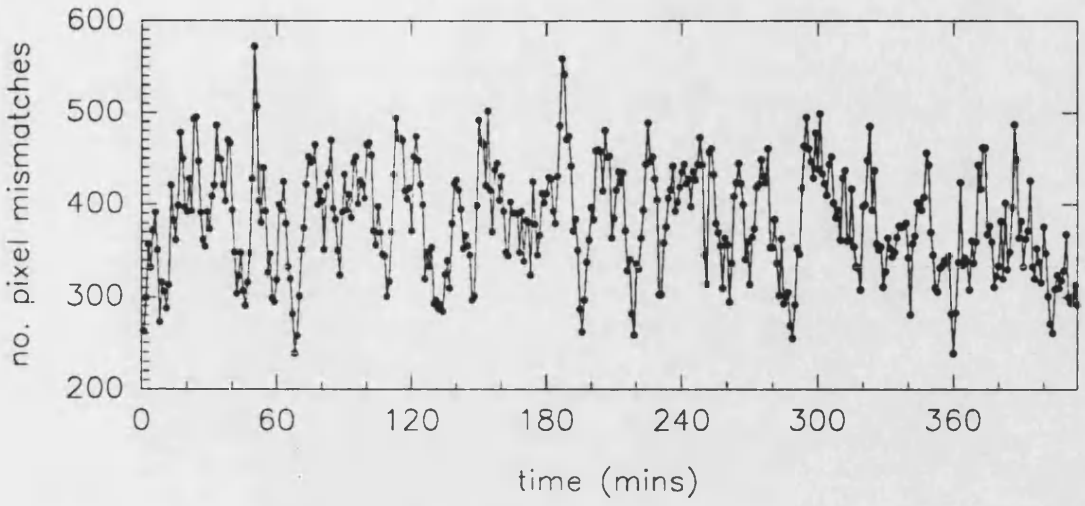

26
28
34
43
no. pixel mismatches
no. pixel mismatches
no. pixel mismatches
no. pixel mismatches
400
350
300
250
200
150
100
50
450
400
350
300
250
200
150
100
50
500
450
400
350
300
250
200
150
100
50
350
300
250
200
150
100
50
0
60
120
180
240
300
360
time (mins)

Sample time series of activity in the whole field of view: Experiments in Chapter 7. Activity is measured as the number of pixel mismatches between consecutive frames taken at one minute intervals in window X0. Graphs present activity in runs and dates as labelled.

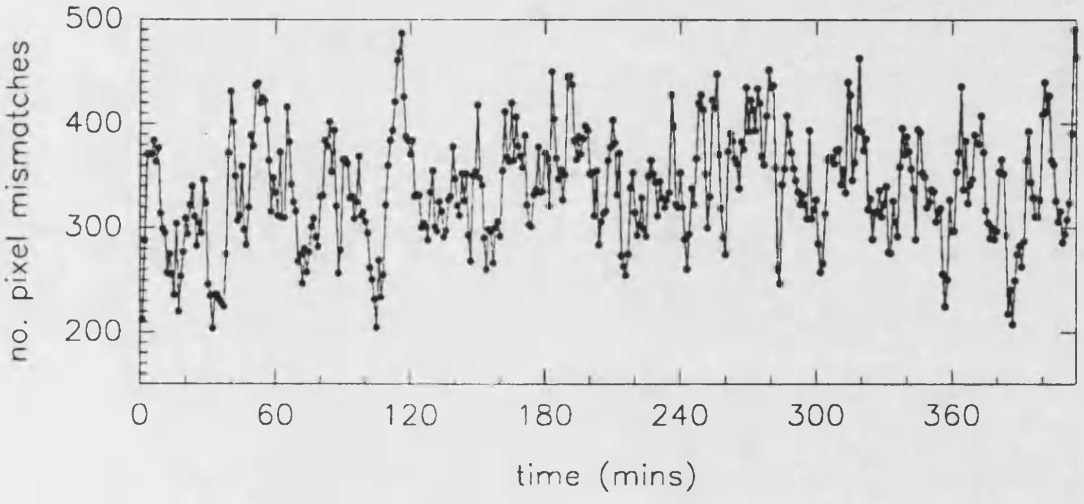
ZIGRUN 01



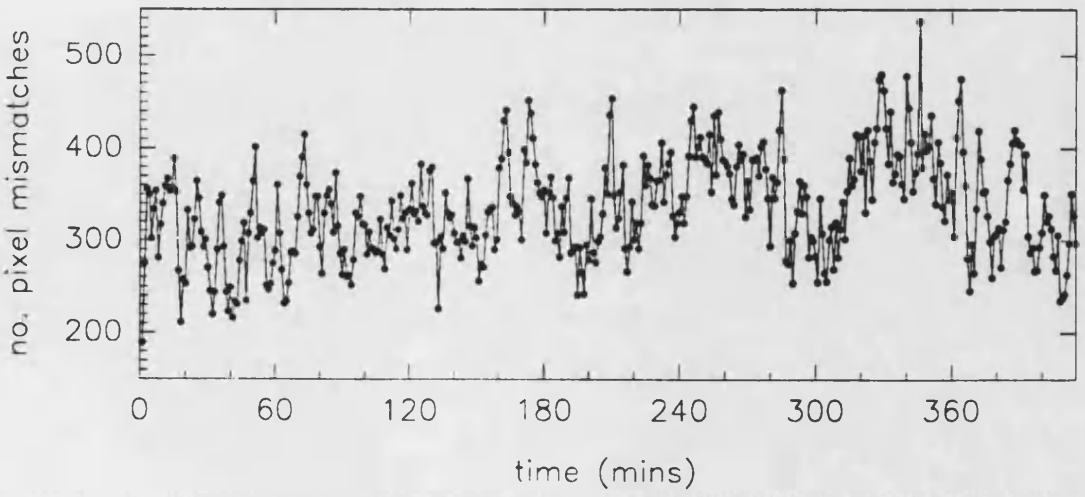
02


600
500
400
300
200
no. pixel mismatches
0
60
120
180
240
300
360
time (mins)

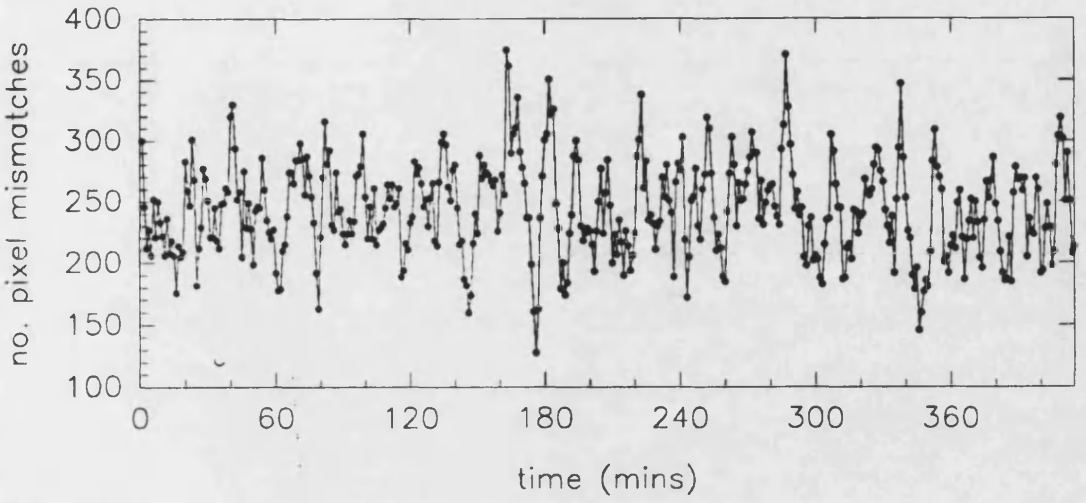
08


500
400
300
200
no. pixel mismatches
0
60
120
180
240
300
360
time (mins)

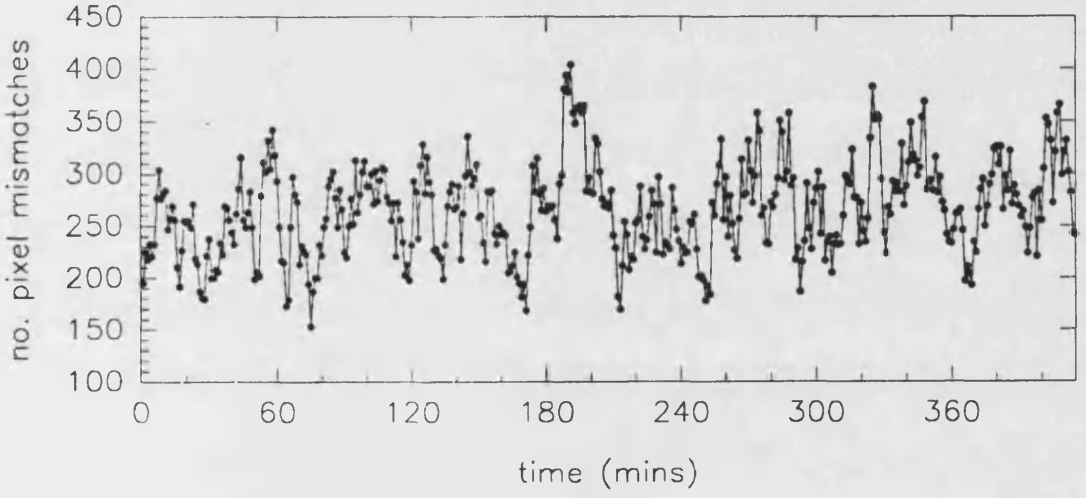
16


500
400
300
200
no. pixel mismatches
0
60
120
180
240
300
360
time (mins)

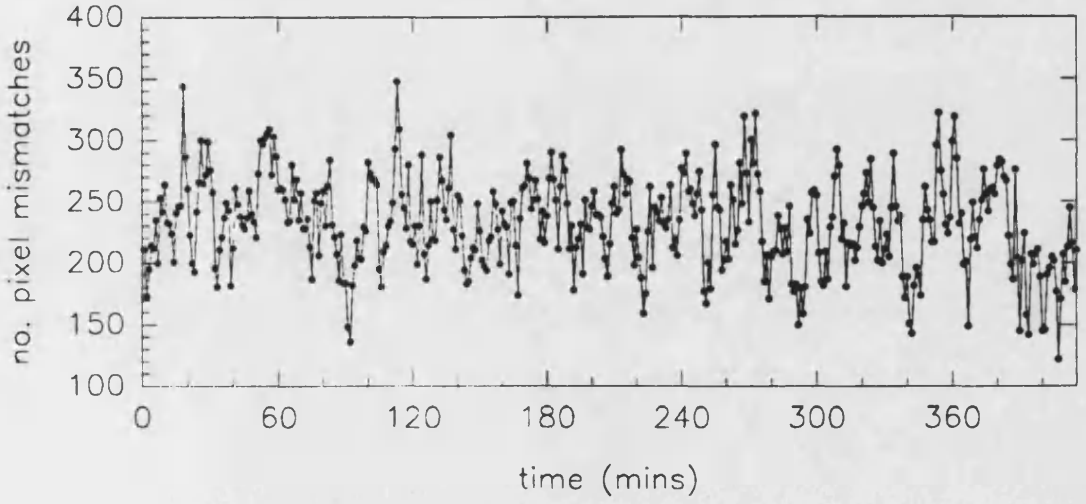
01


no. pixel mismatches
400
350
300
250
200
150
100
0
60
120
180
240
300
360
time (mins)

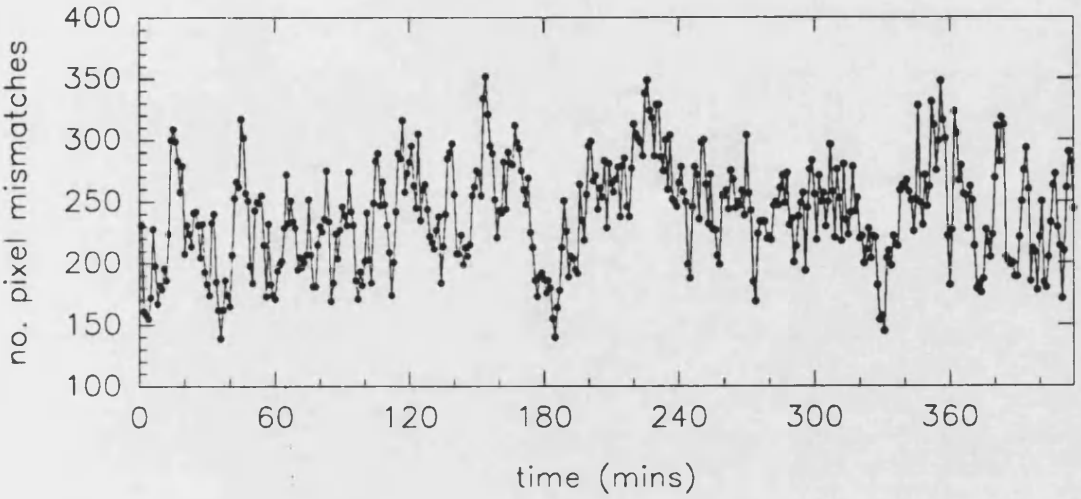
04


no. pixel mismatches
450
400
350
300
250
200
150
100
0
60
120
180
240
300
360
time (mins)

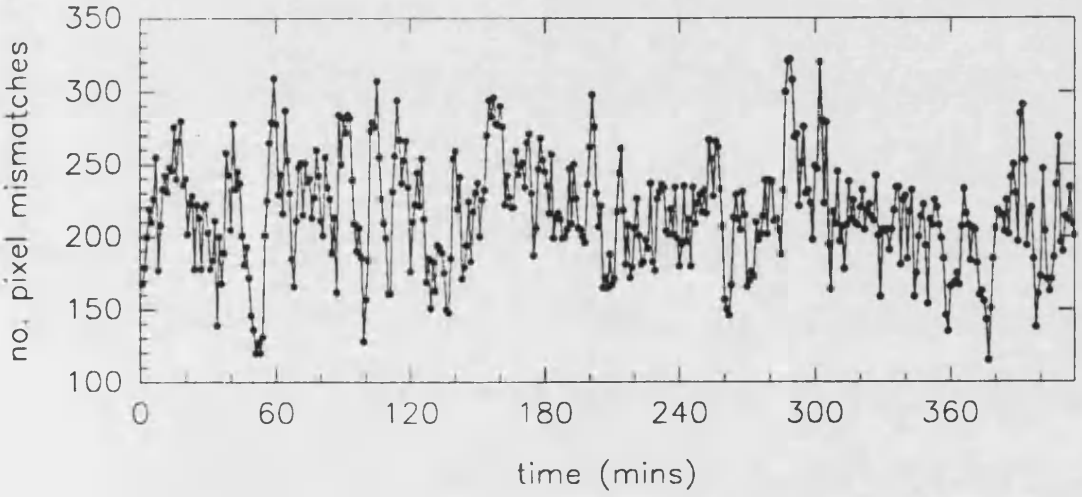
08


no. pixel mismatches
400
350
300
250
200
150
100
0
60
120
180
240
300
360
time (mins)

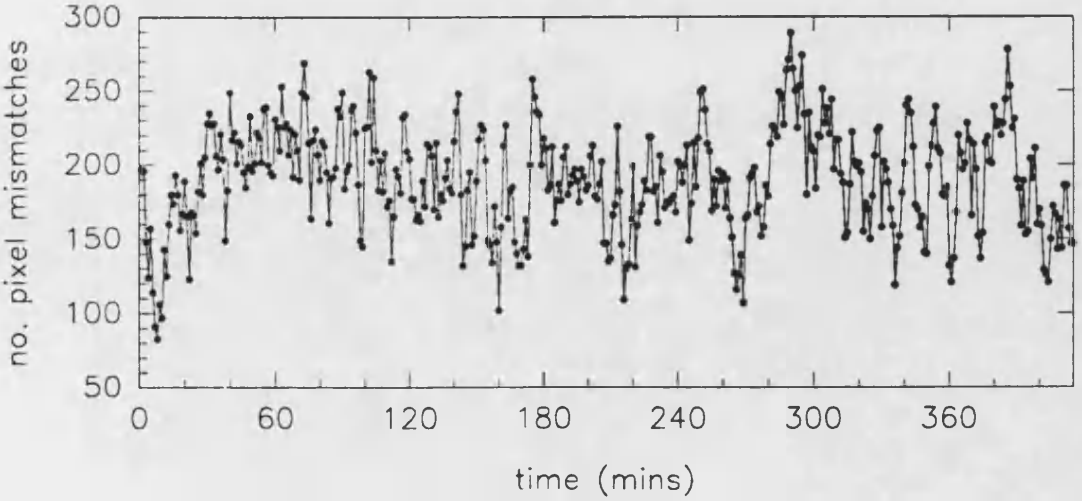
02


no. pixel mismatches
400
350
300
250
200
150
100
0
60
120
180
240
300
360
time (mins)

05

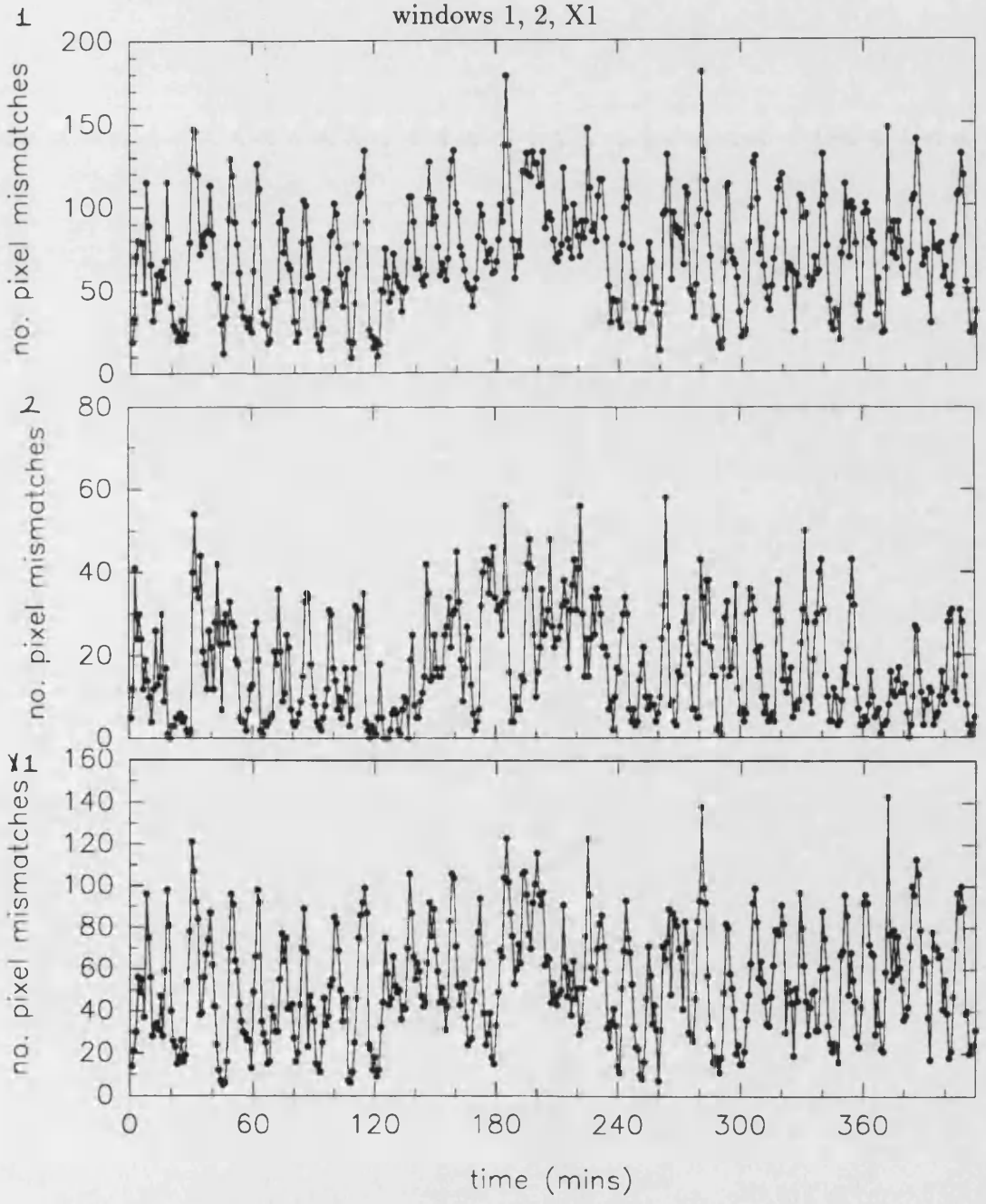

no. pixel mismatches
350
300
250
200
150
100
0
60
120
180
240
300
360
time (mins)

10

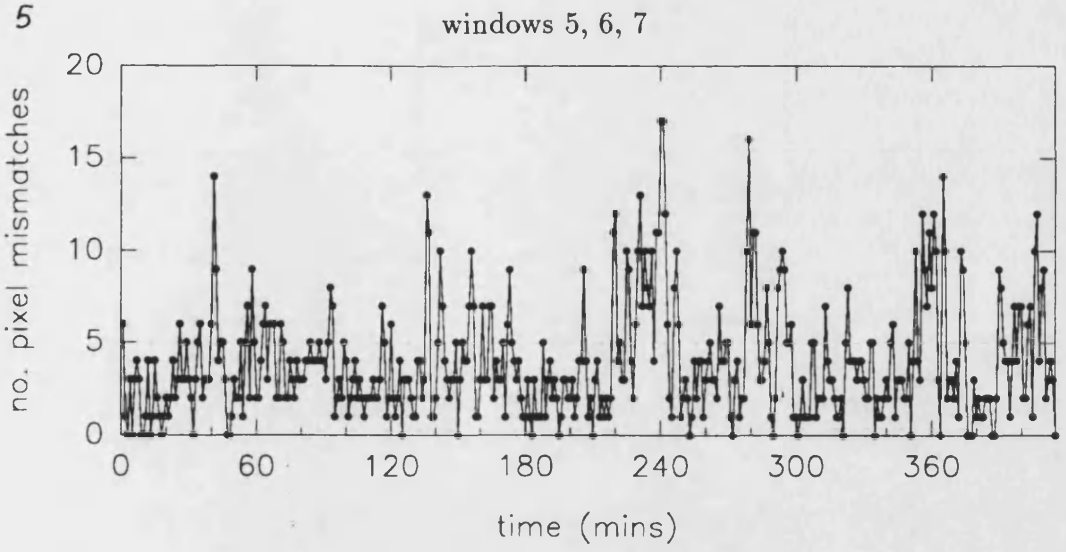

no. pixel mismatches
300
250
200
150
100
50
0
60
120
180
240
300
360
time (mins)

D.2 Time Series: Selected Windows

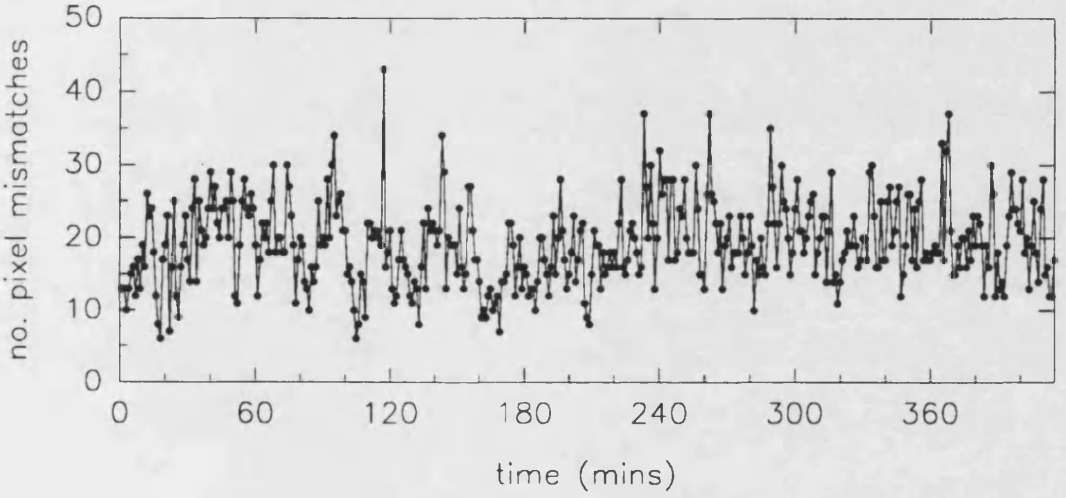
Time series of activity in all windows for day 04 of MIDRUN. Activity is measured as the number of pixel mismatches between consecutive frames taken at one minute intervals in windows (as labelled). Windows are identified in Table 4.2.3



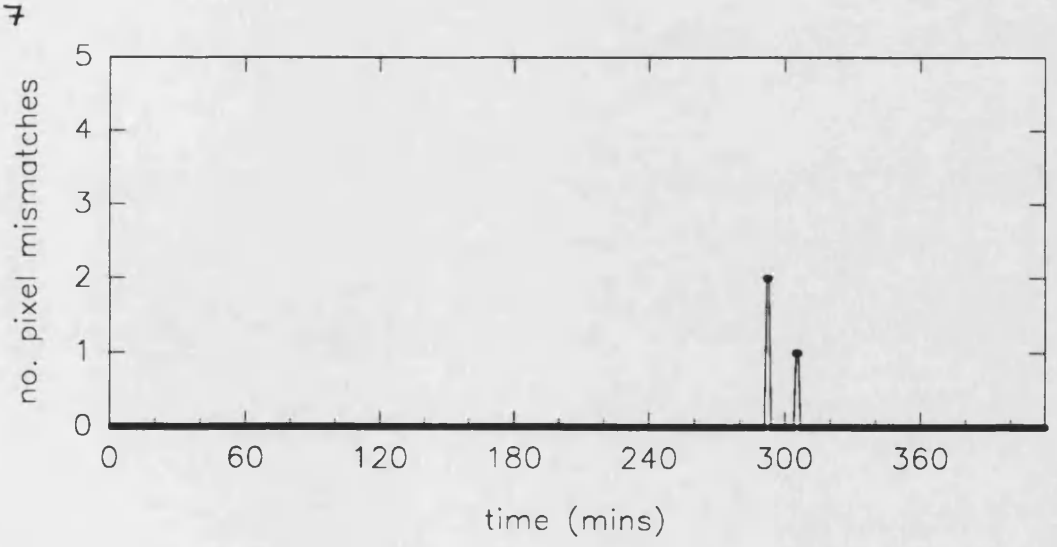
5


windows 5, 6, 7
no. pixel mismatches
0
5
10
15
20
0
60
120
180
240
300
360
time (mins)

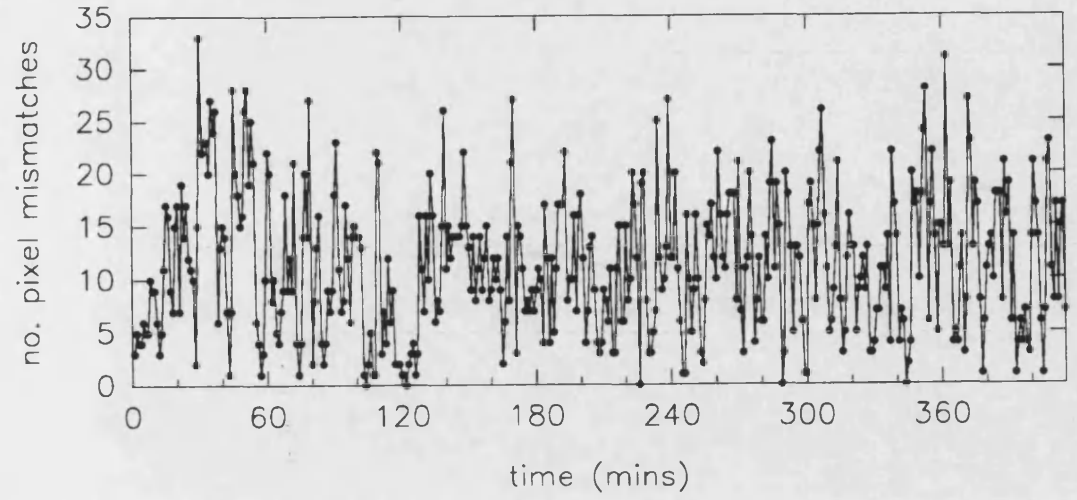
6


no. pixel mismatches
0
10
20
30
40
50
0
60
120
180
240
300
360
time (mins)

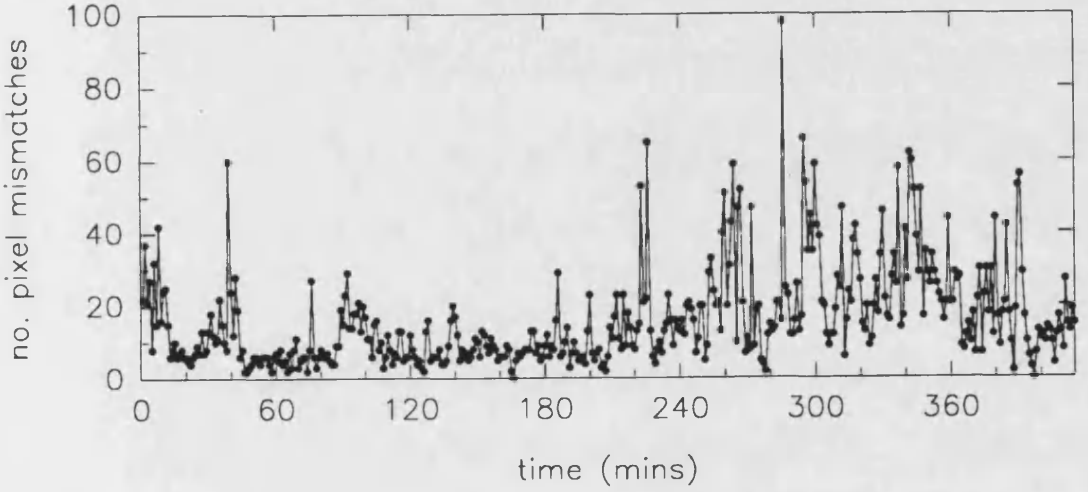
7


no. pixel mismatches
0
1
2
3
4
5
0
60
120
180
240
300
360
time (mins)

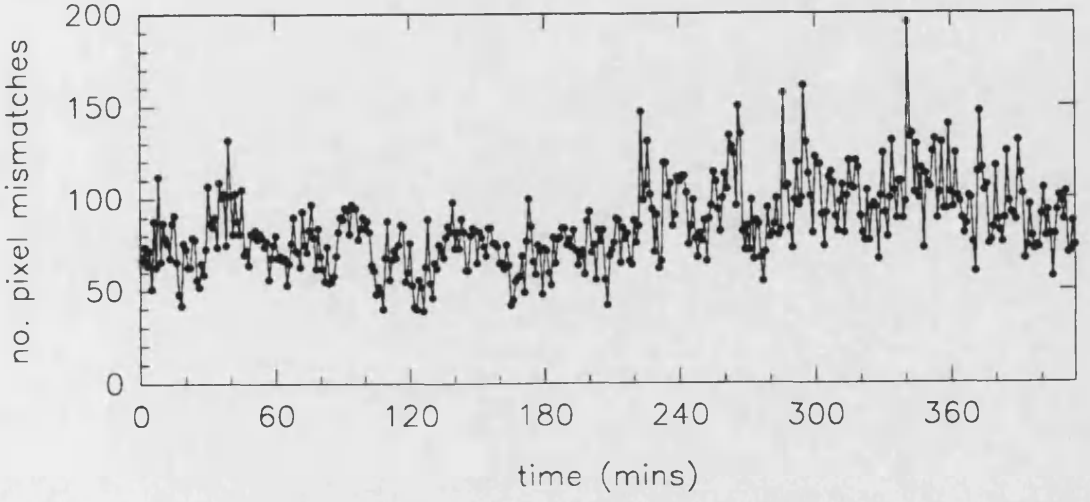
8


windows 8, 9, X2
no. pixel mismatches
0
5
10
15
20
25
30
35
0
60
120
180
240
300
360
time (mins)

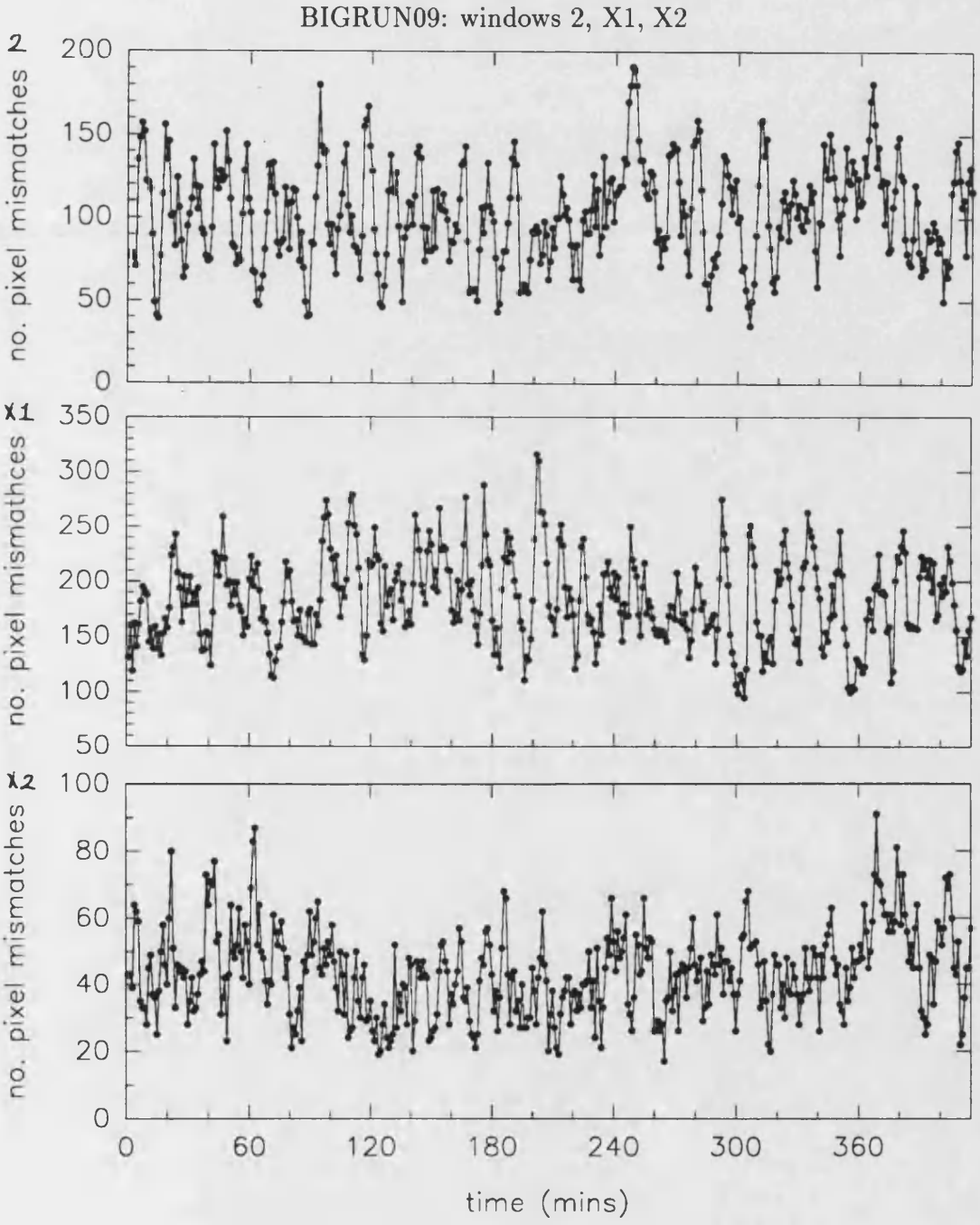
9

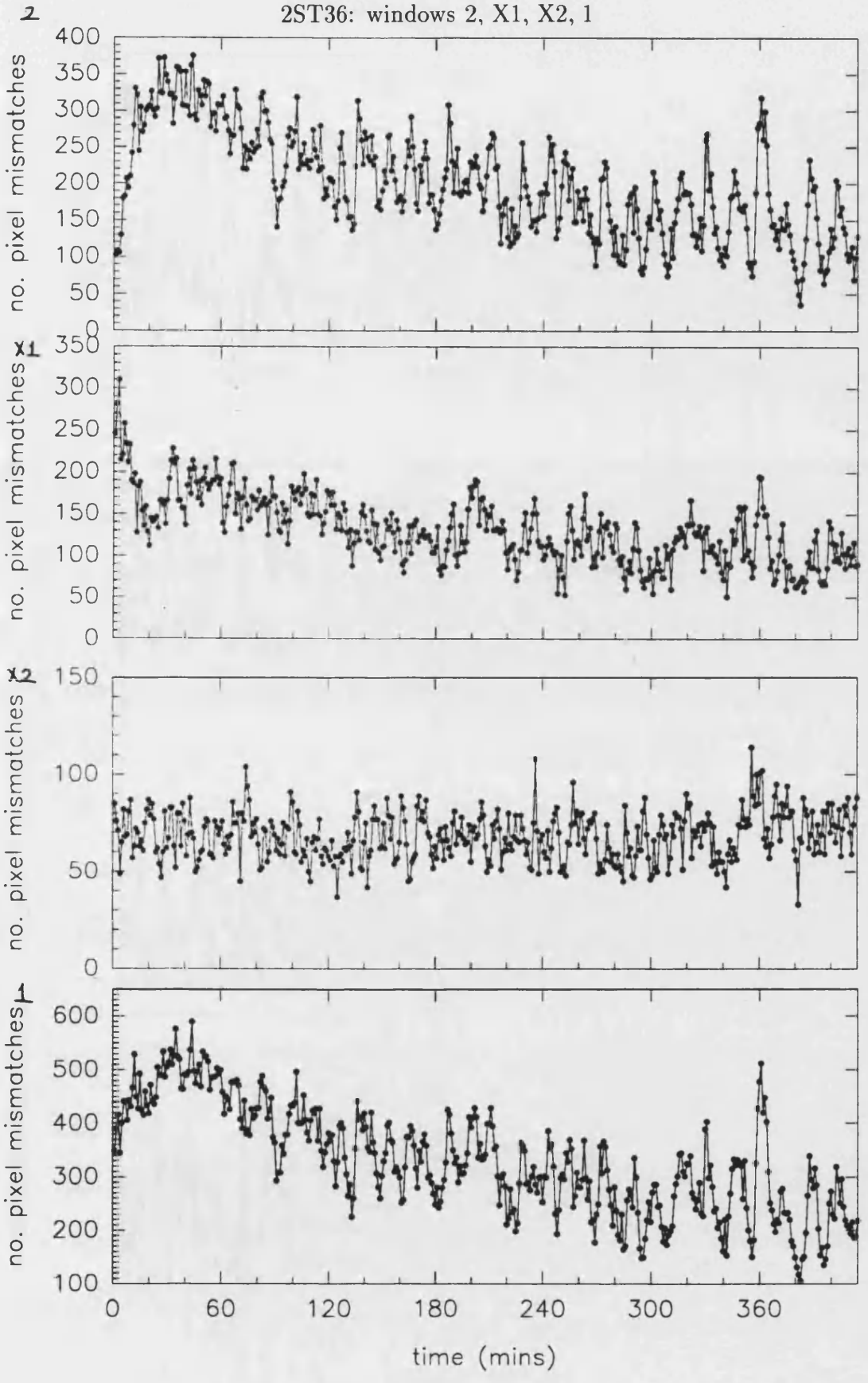

no. pixel mismatches
0
20
40
60
80
100
0
60
120
180
240
300
360
time (mins)

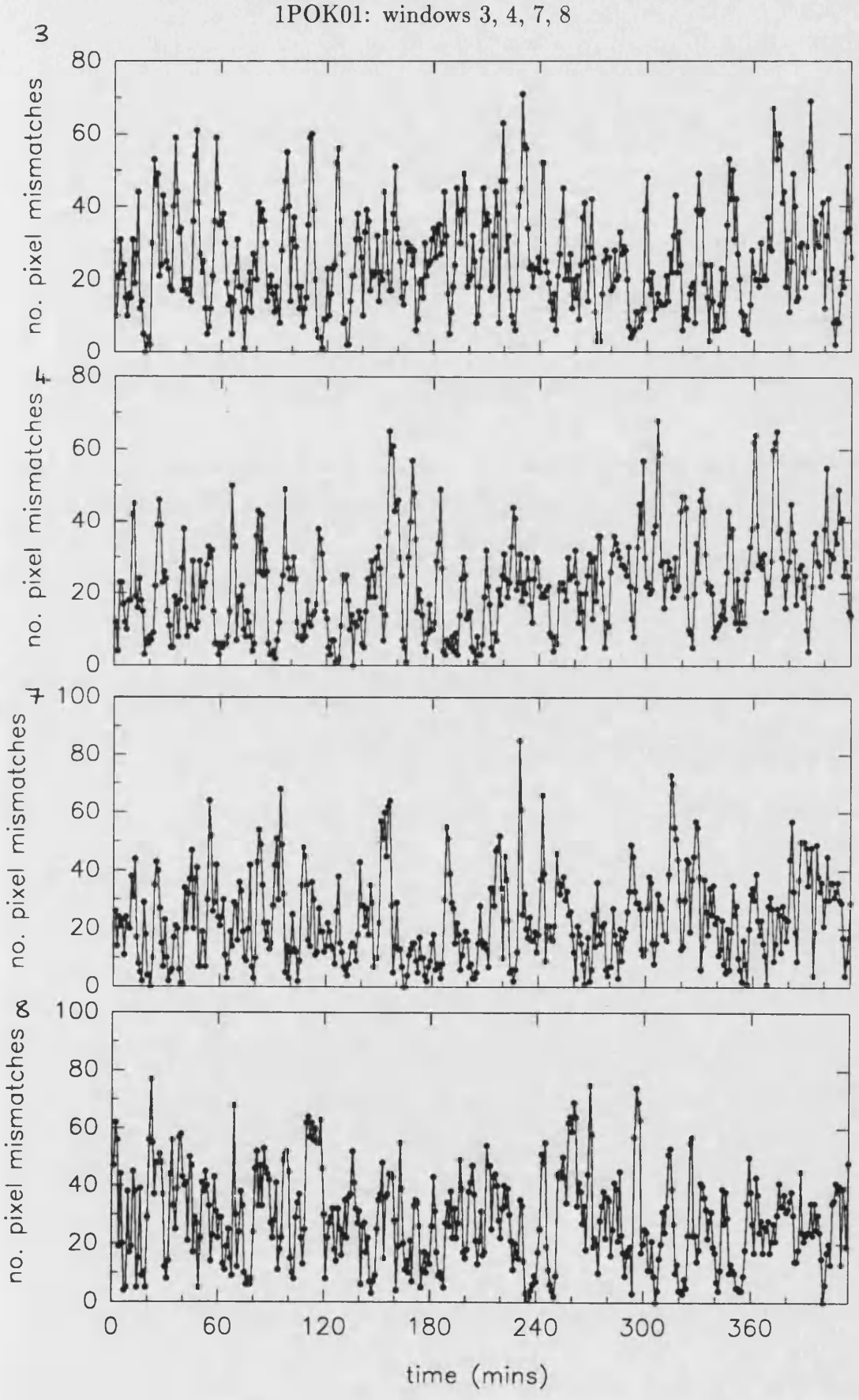
10


no. pixel mismatches
0
50
100
150
200
0
60
120
180
240
300
360
time (mins)

Sample time series of activity in windows for experiments in Chapters 4, 6 and 7. Activity is measured as the number of pixel mismatches between consecutive frames taken at one minute intervals in windows and run days (as labelled). Windows are identified in Table 4.2.3



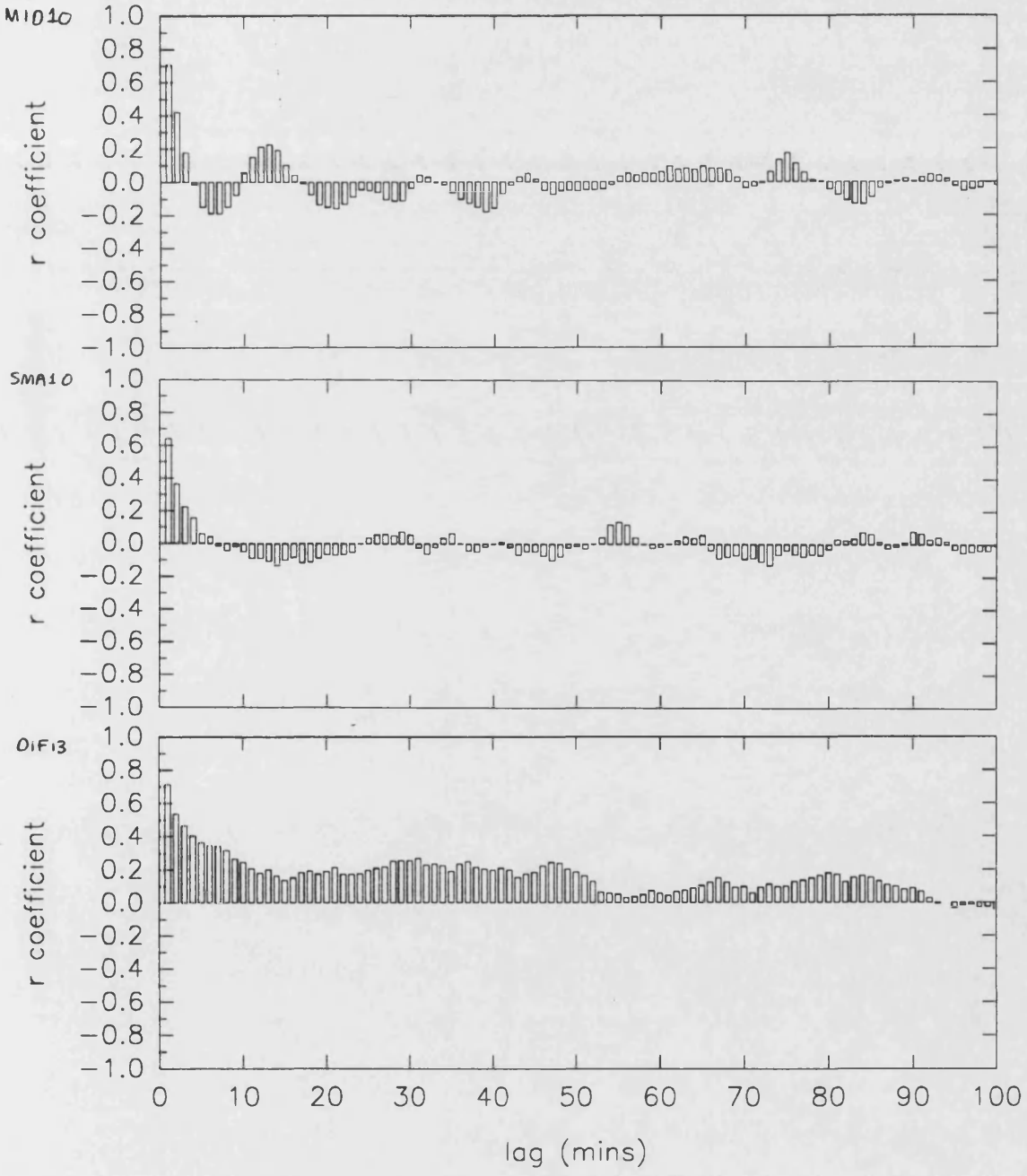

2ST36: windows 2, X1, X2, 1
2
no. pixel mismatches
400
350
300
250
200
150
100
50
0
X1
no. pixel mismatches
350
300
250
200
150
100
50
0
X2
no. pixel mismatches
150
100
50
0
1
no. pixel mismatches
600
500
400
300
200
100
0
60
120
180
240
300
360
time (mins)


1POK01: windows 3, 4, 7, 8
3
4
7
8
no. pixel mismatches
0
20
40
60
80
100
0
60
120
180
240
300
360
time (mins)

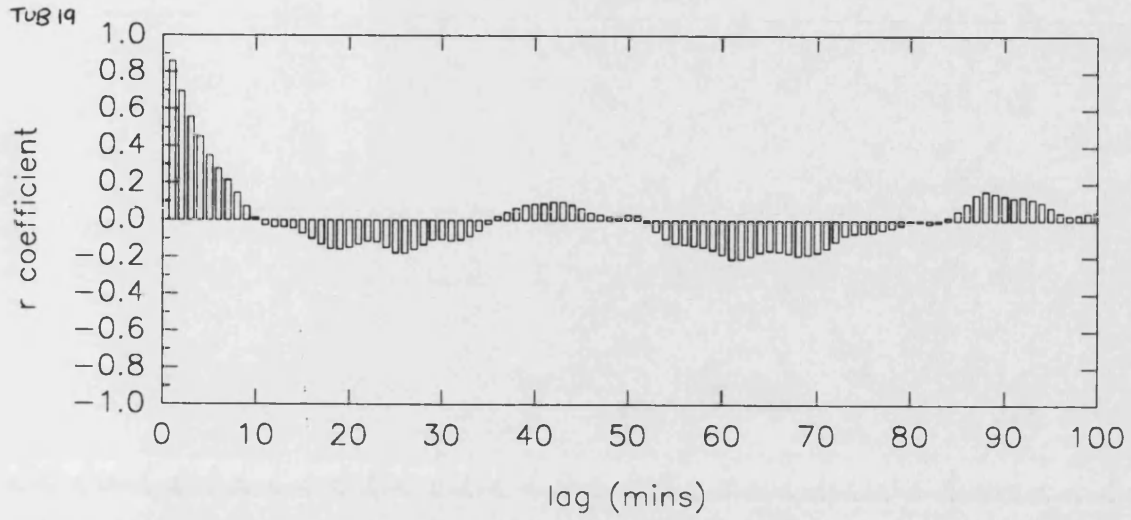
D.3 Autocorrelograms

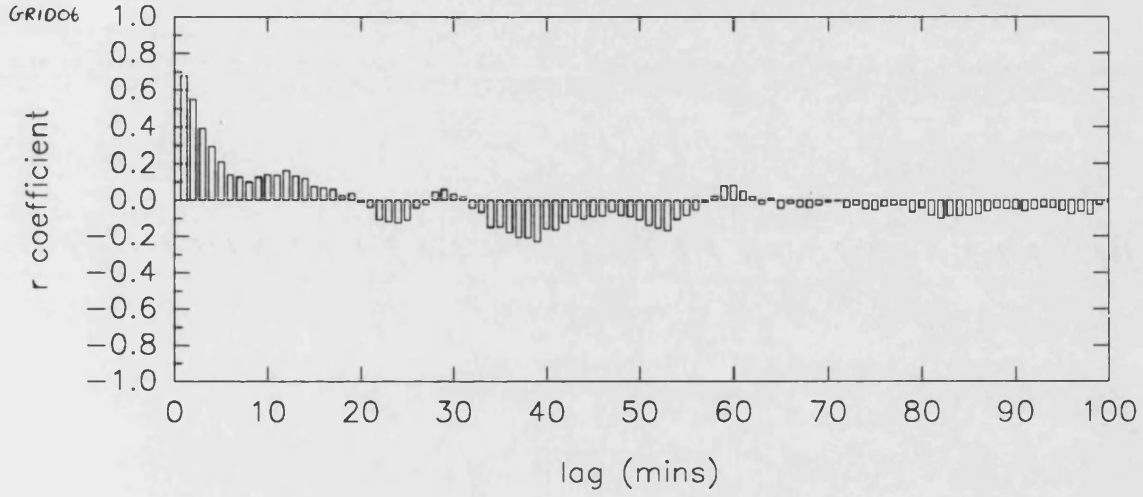
Sample autocorrelograms of daily activity time series for Chapters 4, 6 and 7. For autocorrelation calculations see Section A.1. Runs and dates as labelled.

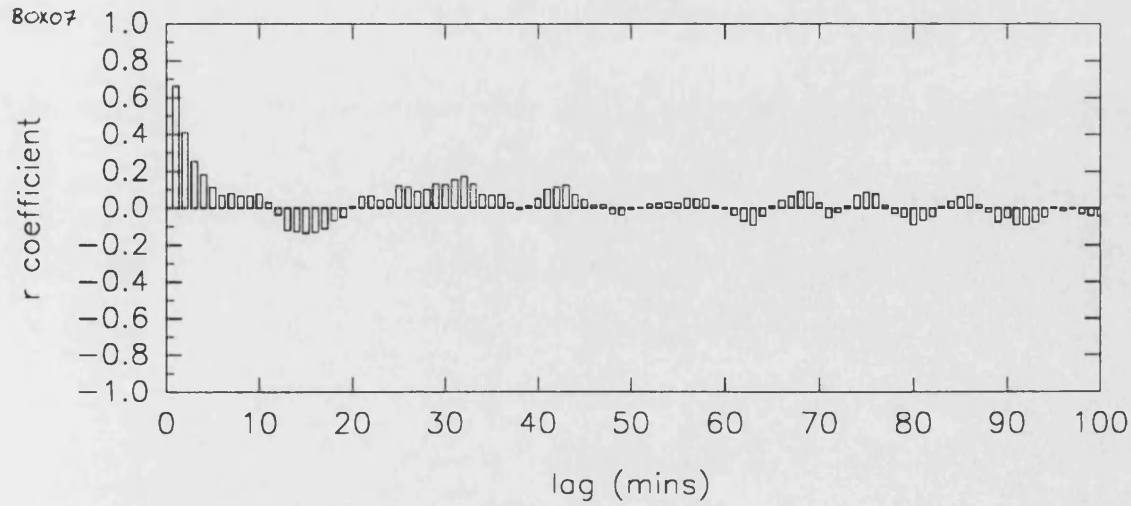
MIDRUN10, SMARUN10, DIFRUN13



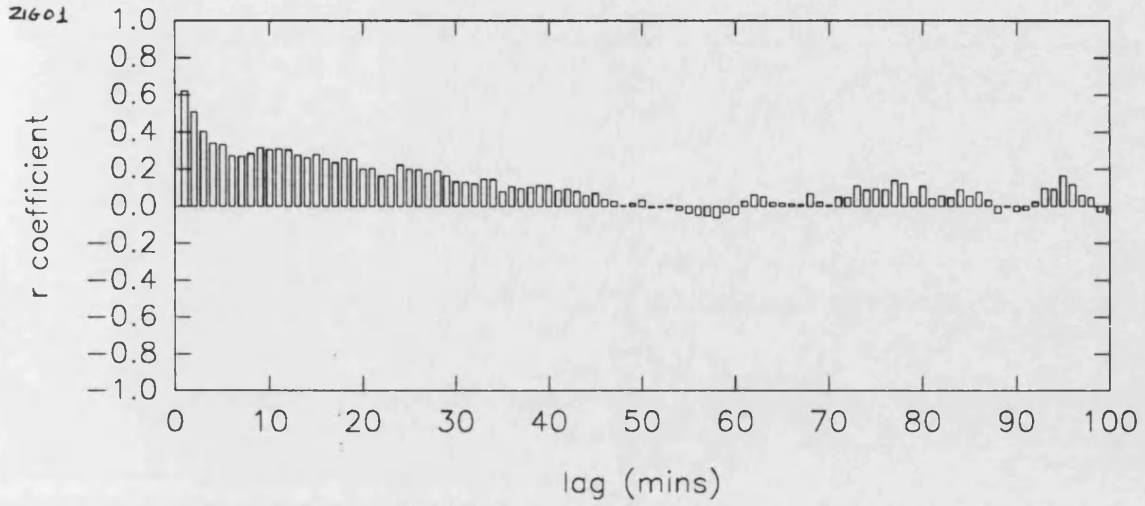
TUBRUN19, GRIDR06, BOX07

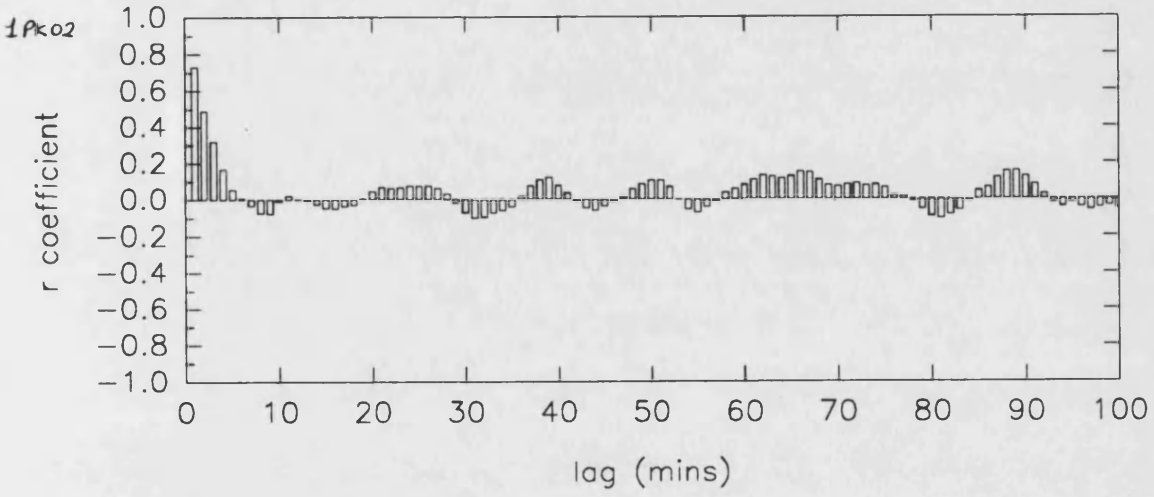


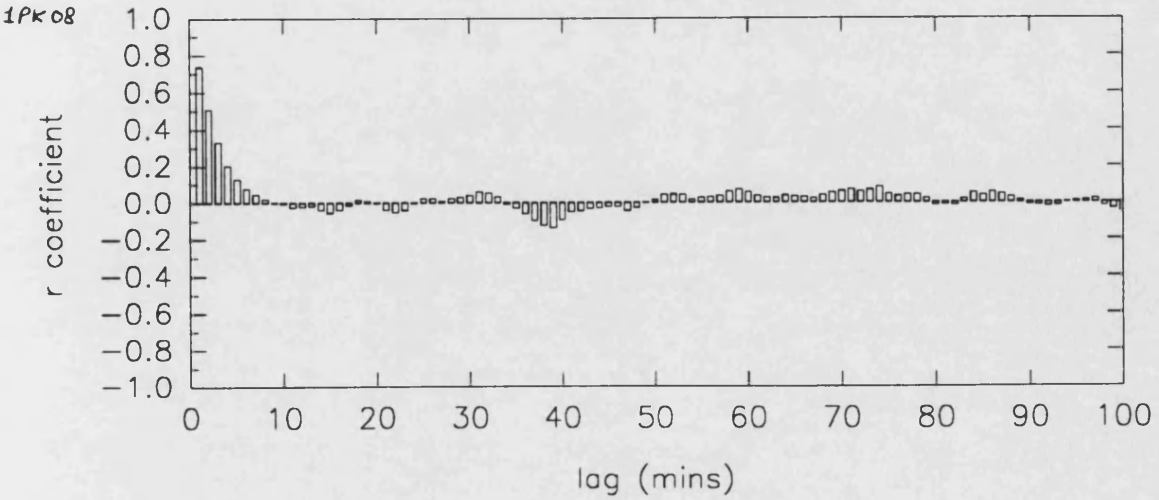




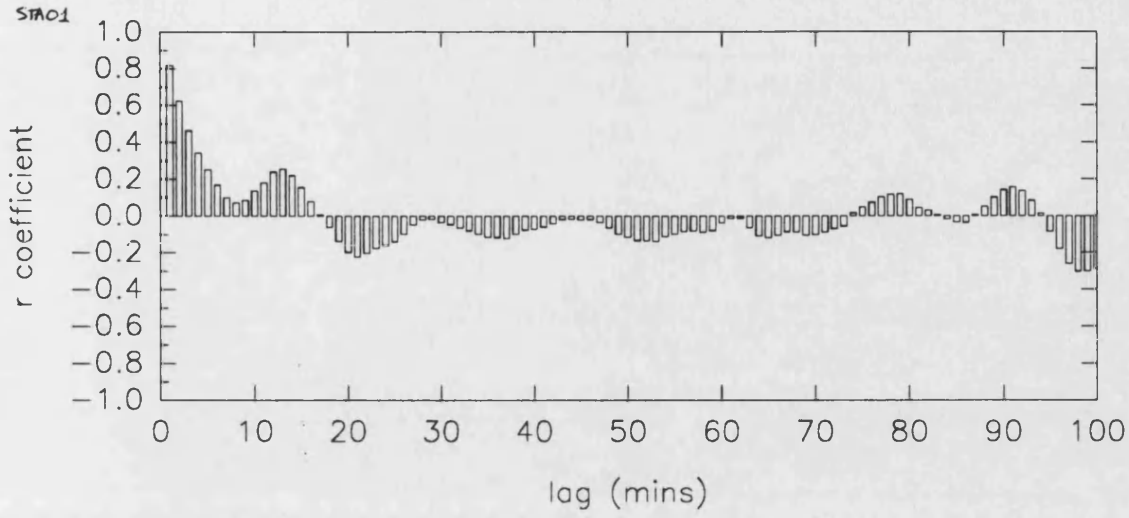
ZIGRUN01, 1POK02, 1POK08

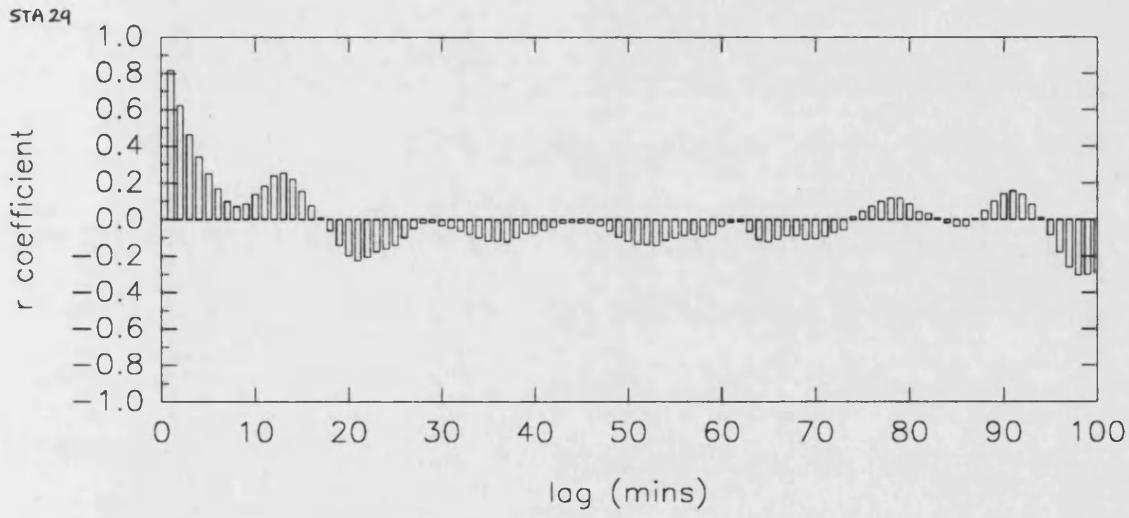


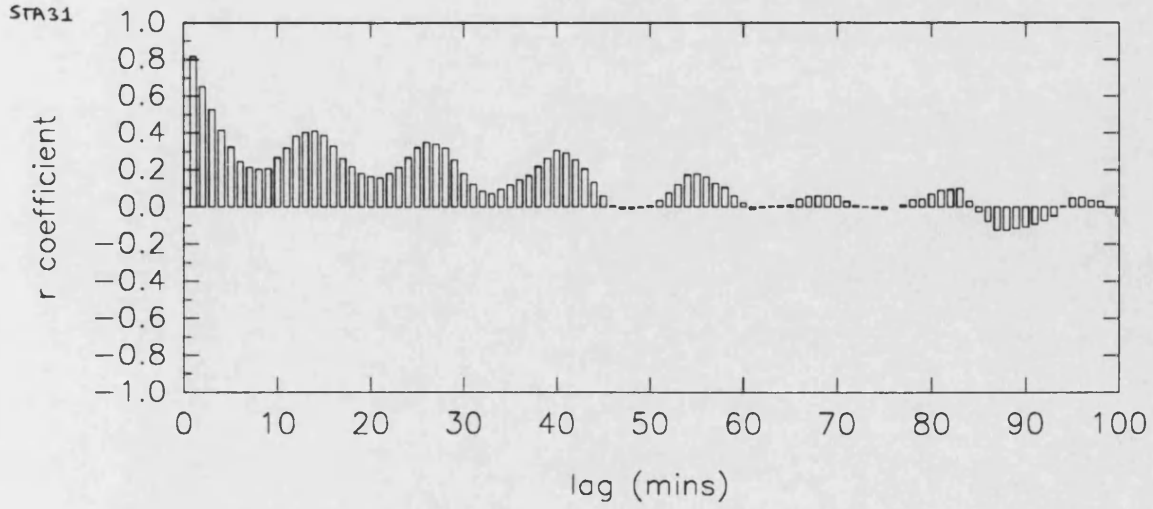




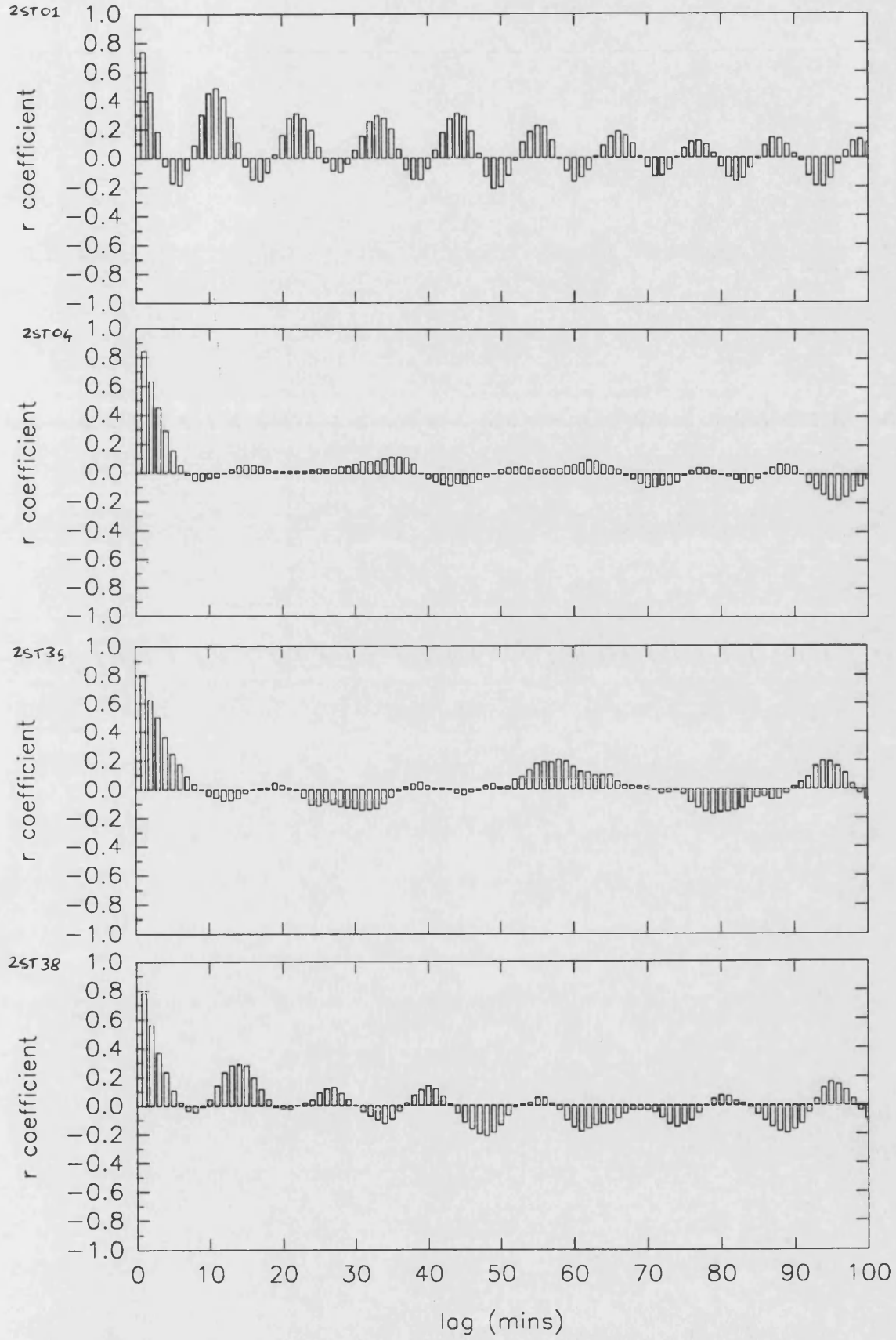
STA01, STA29, STA31





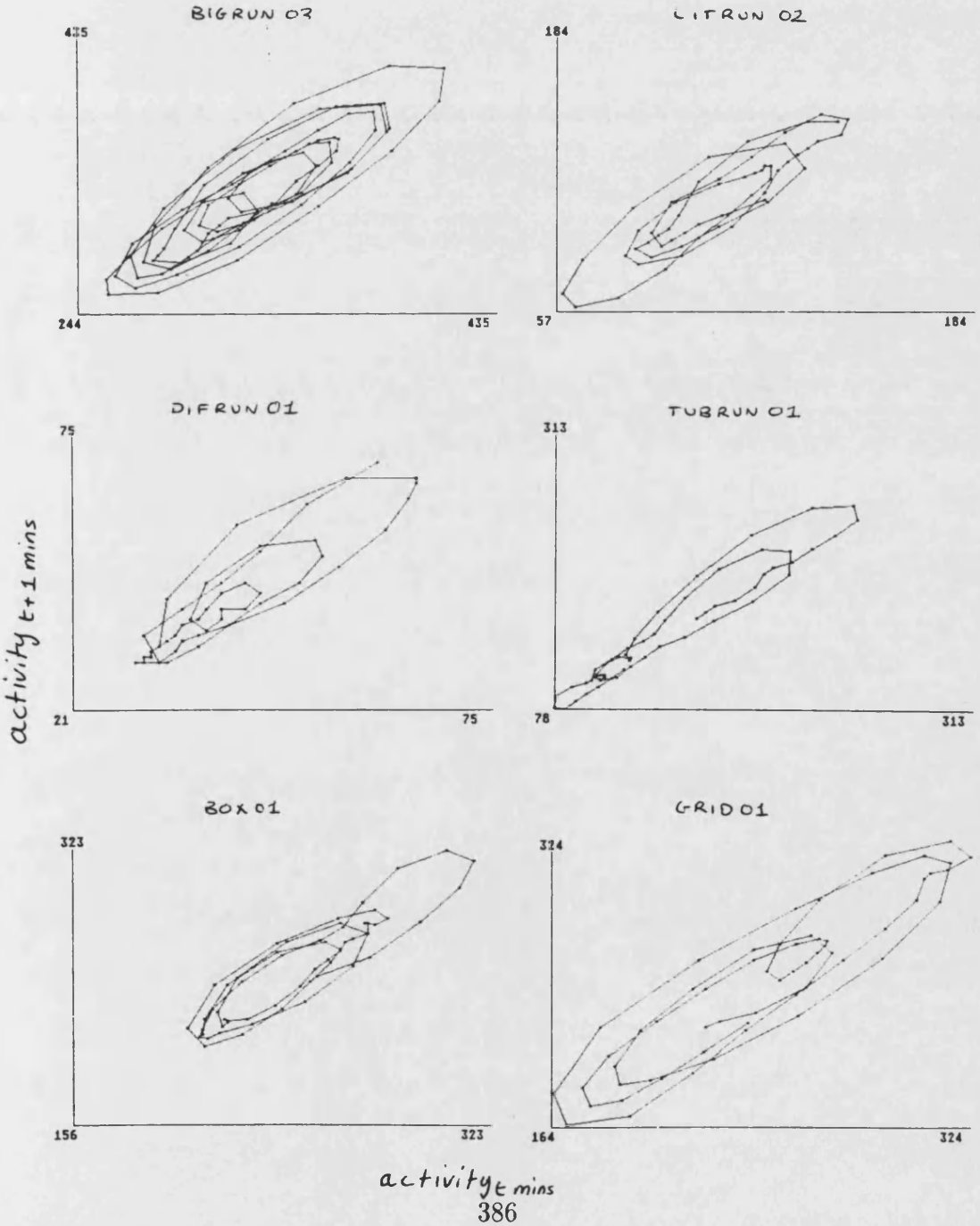


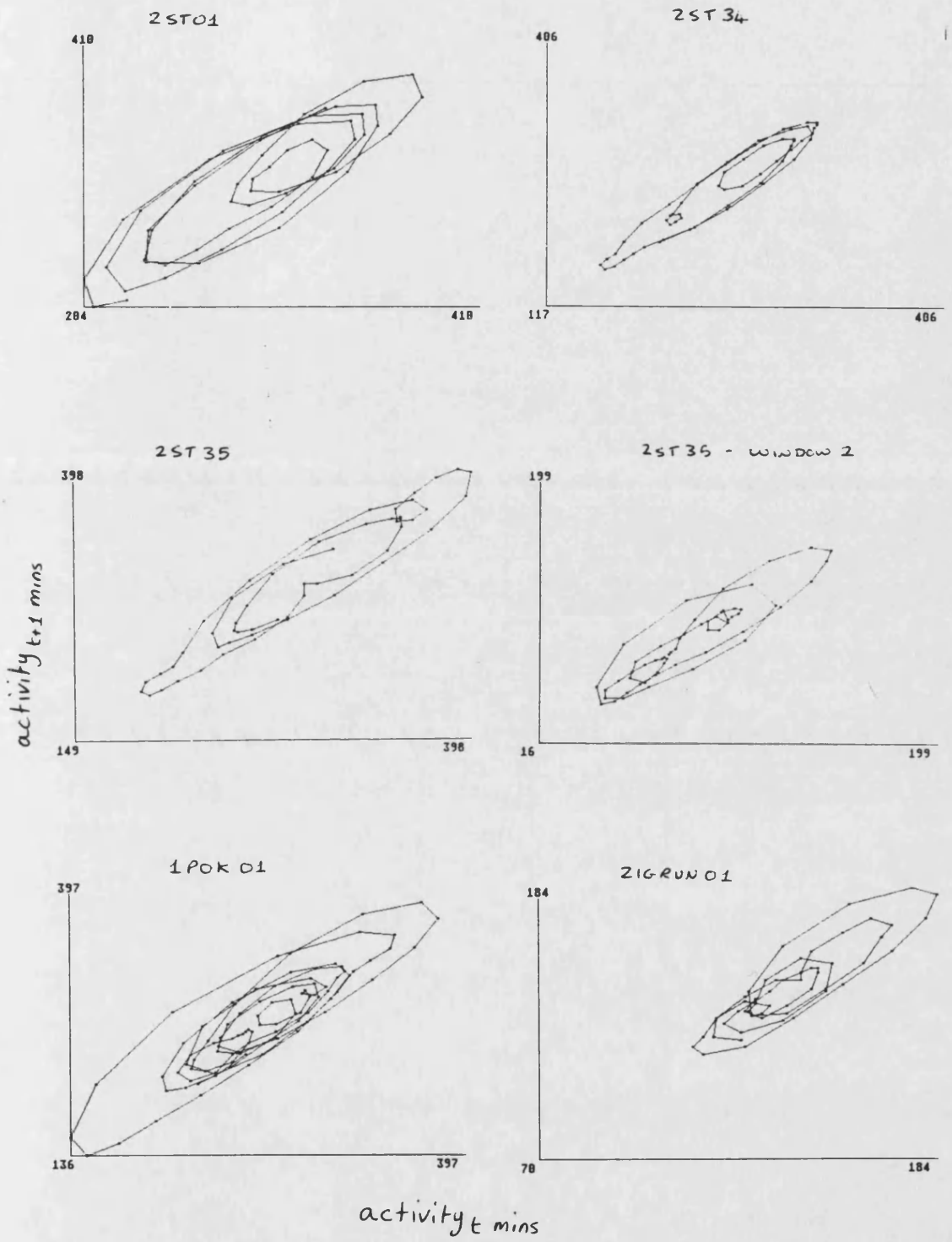
2ST01, 2ST04, 2ST35, 2ST38



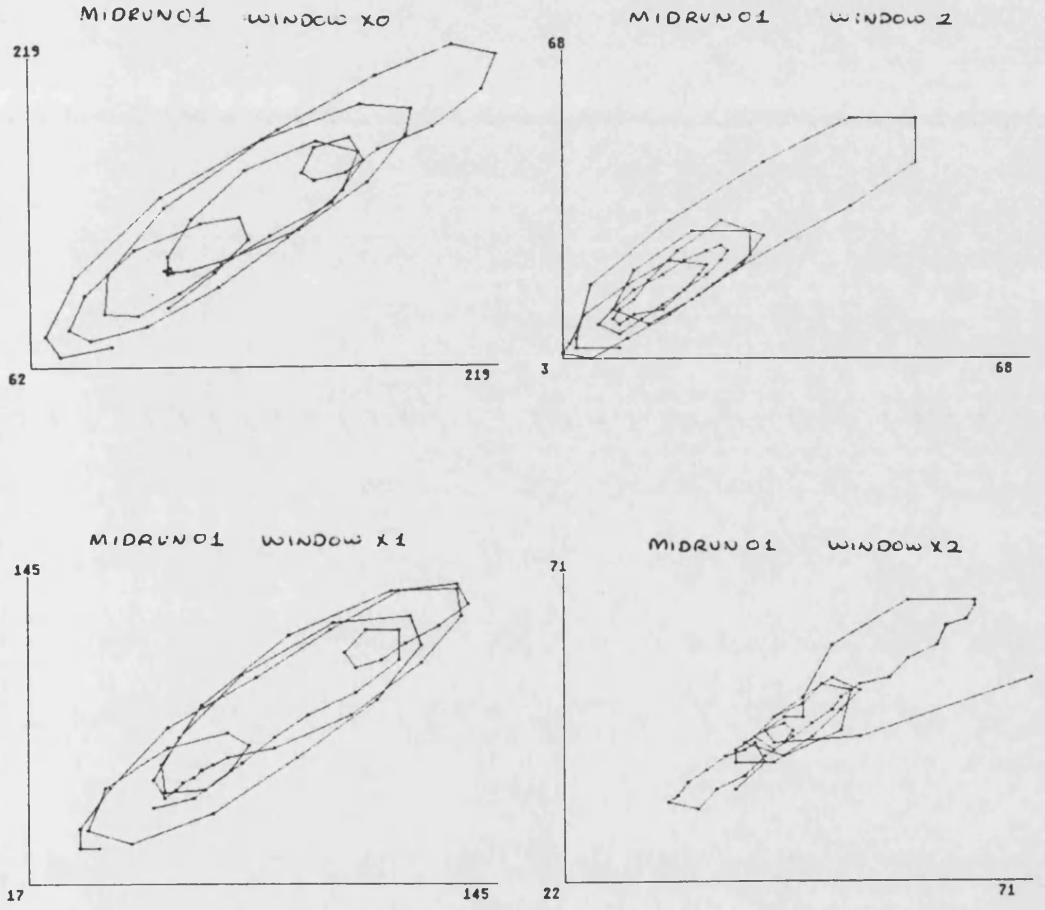
D.4 Sample Return Maps

Sample first return maps of activity time series data for Chapters 4, 6 and 7. Activity at time t (horizontal axis) is plotted against activity at time $t+1$ (vertical axis), activity is measured as number of pixel mismatches between consecutive frames at 1 minute intervals in window X0. Return maps are presented for runs and days as labelled.



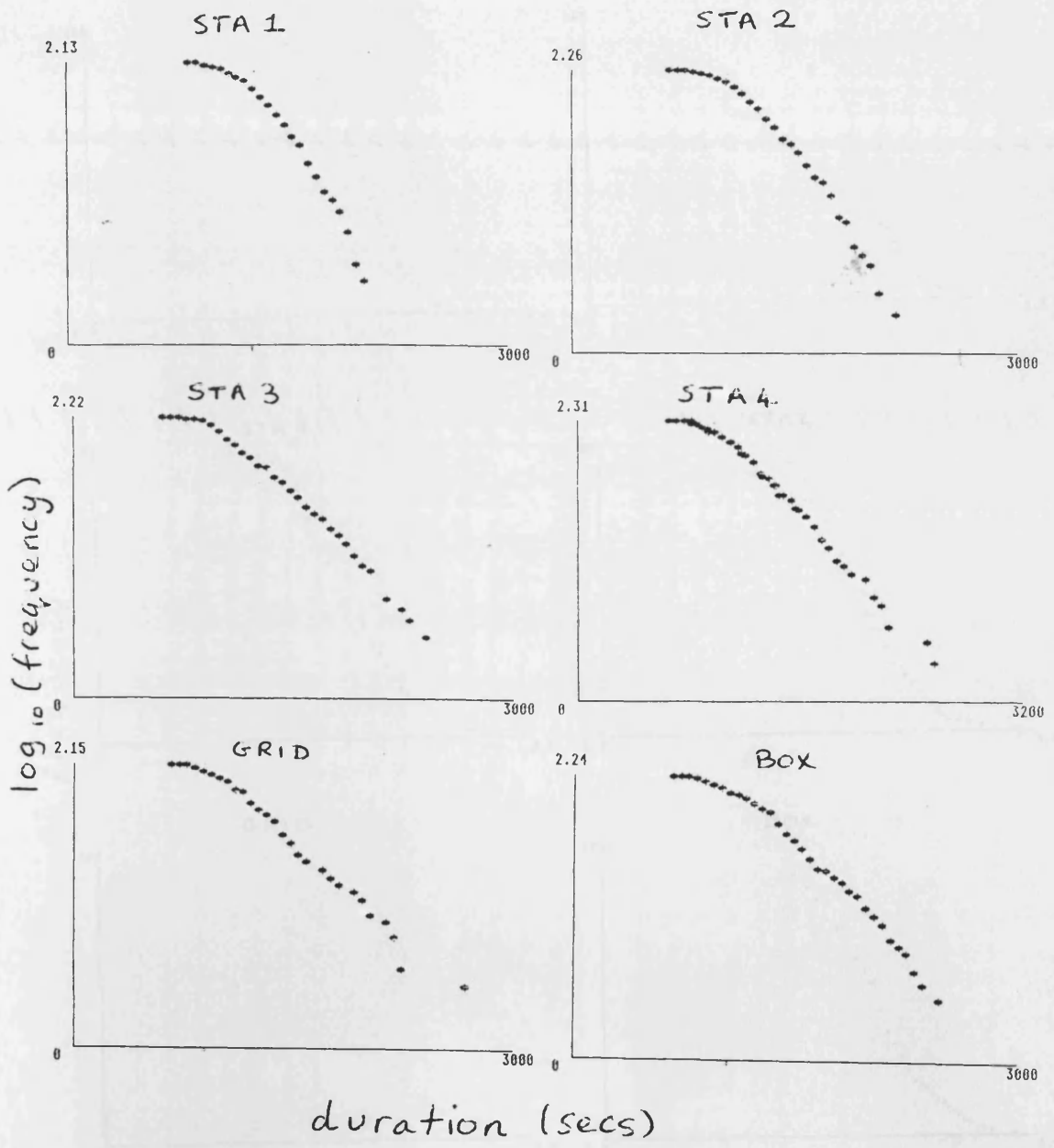

2ST01
418
284
418
2ST34
406
117
406
2ST35
398
149
398
2ST35 - WINDOW 2
199
16
199
activity t+1 mins
1POK01
397
136
397
2IGRUN01
184
78
184
activity t mins

Sample first return maps of activity time series data for MIDRUN. Activity at time t (horizontal axis) is plotted against activity at time $t + 1$ (vertical axis), activity is measured as number of pixel mismatches between consecutive frames at 1 minute intervals in windows as labelled. Return maps are presented for runs and days as labelled. The graphs show activity cycling for the time interval 0 to 60 minutes in the experiment.

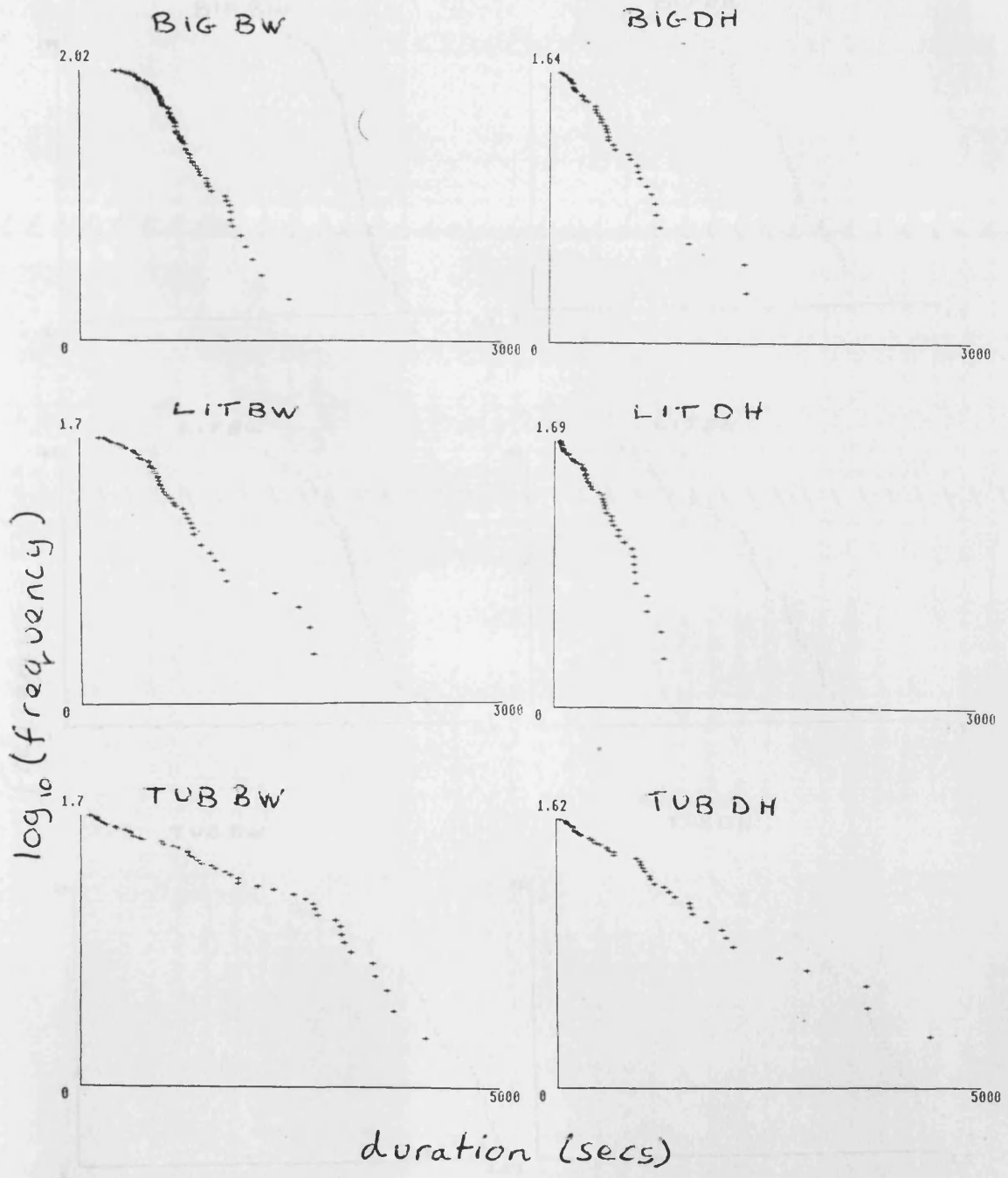


D.5 Log Survivorship Graphs

Sample log survivorship plots of cycle length for Chapters 6 and 7. Each point shows the number of cycles that were longer than the duration on the horizontal axis. Cycles were measured by the trough location procedure applied to activity time series from window X0. Runs as labelled.



Sample log survivorship plots of inactive bout length for individuals as measured in Chapter 5. Each point shows the number of bouts that were longer than the duration on the horizontal axis. Individuals were grouped into two task groups: BW, brood workers; DH, door hangers. Runs and task groups as labelled.

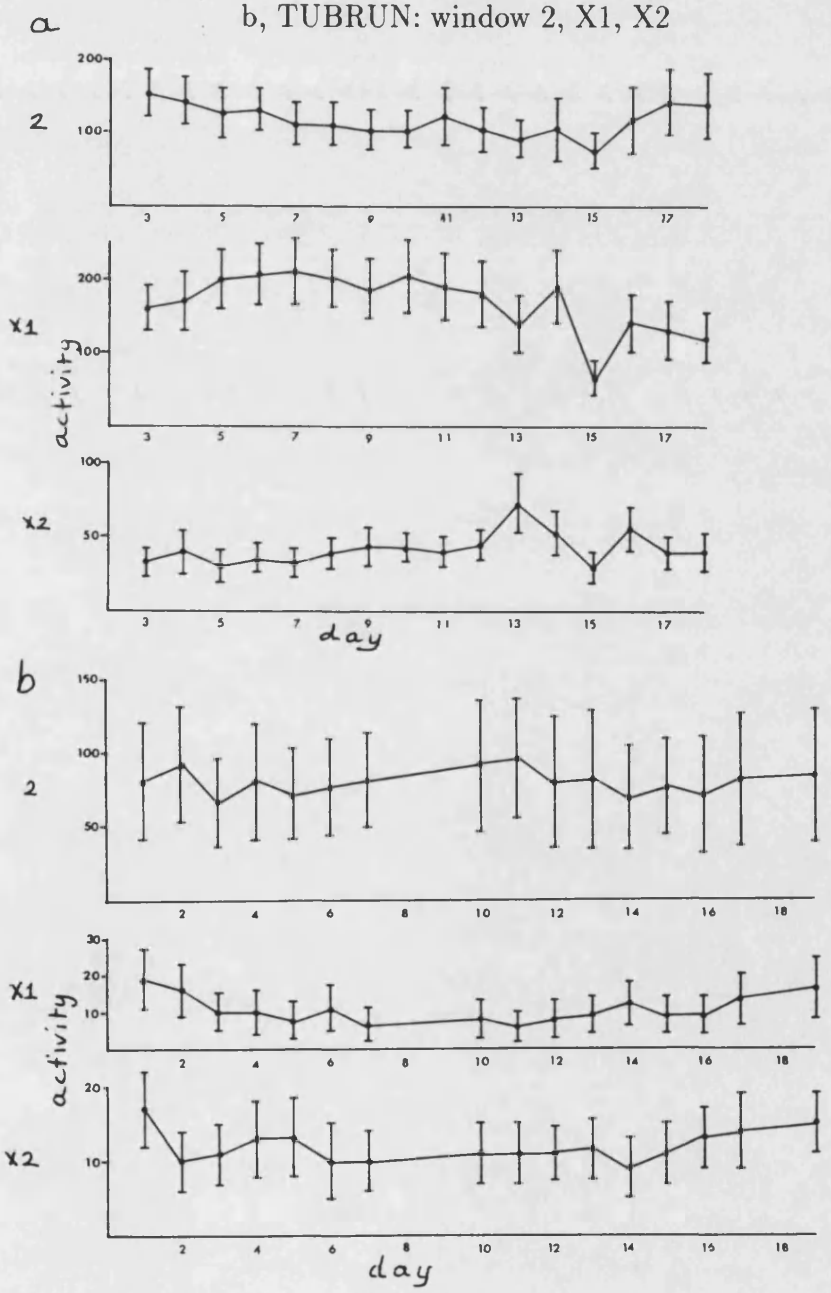


D.6 Mean Activity Level

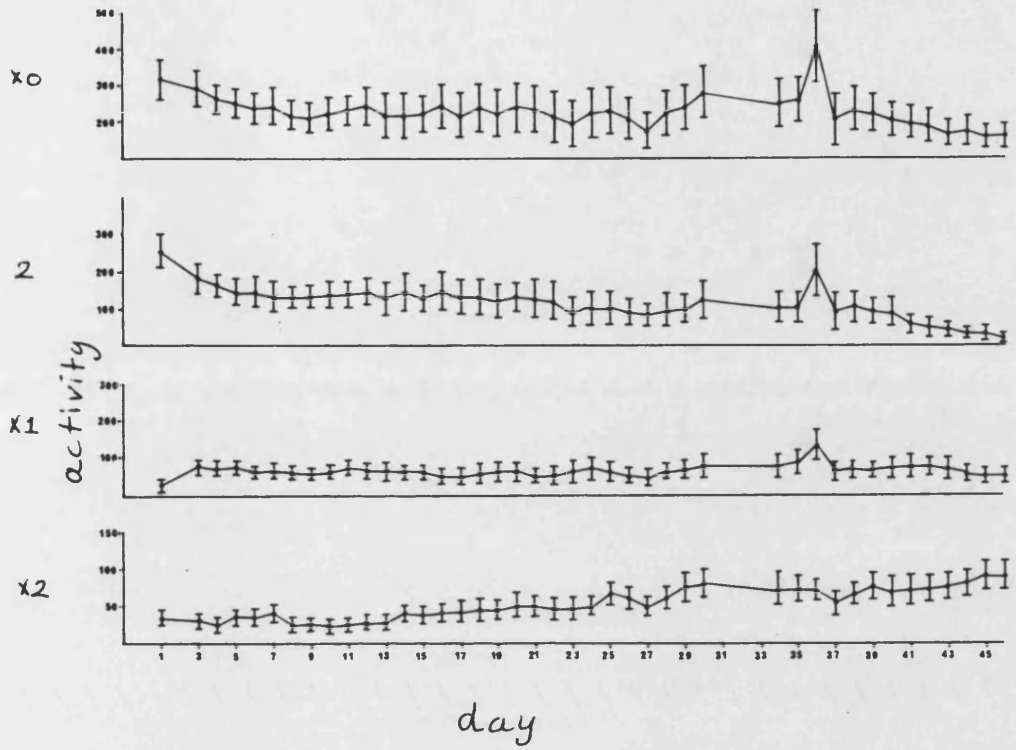
Mean activity level for whole runs for various windows. Activity is measured as the number of pixel mismatches. For runs and windows (as labelled), mean activity level and standard deviation are plotted for each day.

a, BIGRUN: window 2, X1, X2

b, TUBRUN: window 2, X1, X2



2ST: window X0, 2, X1, X2



D.7 Activity in the Linear Nest

Correlations between activity levels within ZIGRUN. a, sample seg ment of nest from ZIG01. Significant correlations are shown as lines between the first 12 windows closest to the nest entrance (the nest was divided into a total of 40 such windows). b, strong correlations within the whole nest. The forty windows were grouped into 8 consecutive sets of 5 neighbouring windows each, as indicated by numbered circles. Lines indicate strong correlations between sets of windows, judged using the method described in Section A.6. Data for ZIG01.

In both cases, Pearson product moment correlations were calculated between activity time series at 10 second resolution. Lines above the window (boxes/circles) indicate positive correlations, those below indicate negative correlations.